

CONTINUATION APPLICATION
OF

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FOR
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ON
GENETIC TEST TO DETERMINE NON-RESPONSIVENESS
TO STATIN DRUG TREATMENT

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GENETIC TEST TO DETERMINE NON-RESPONSIVENESS TO
STATIN DRUG TREATMENT

BACKGROUND OF THE INVENTION

Throughout this application various publications are referenced within parentheses. The
5 disclosures of these publications in their entirieties are hereby incorporated by reference in this application
in order to more fully describe the state of the art to which this invention pertains.

1. THE FIELD OF THE INVENTION

This invention relates to the medical arts. In particular, it relates to the field of genetic testing
methods and diagnostic kits.

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2. DISCUSSION OF THE RELATED ART

Statin drugs-- the most potent lipid-lowering agents currently available-- are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. They include lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin. All these statin drugs share a common mechanism
15 of action and have similar toxicity profiles. (E. von Kreutz and G. Schluter, *Preclinical safety evaluation of cerivastatin, a novel HMG-CoA reductase inhibitor*, Am. J. Cardiol. 82(4B):11J-17J [1998]; A.G. Ollson [1998]).

The statin drugs are effective in reducing the primary and secondary risk of coronary artery disease
and coronary events, such as heart attack, in middle-aged and older men and women (under 76 years), in
20 both diabetic and non-diabetic patients, and are often prescribed for patients with hyperlipidemia. (A.G. Ollson, *Addressing the challenge*, Eur. Heart J. Suppl. M:M29-35 [1998]; M. Kornitzer, *Primary and secondary prevention of coronary artery disease: a follow-up on clinical controlled trials*, Curr. Opin. Lipidol. 9(6):557-64 [1998]; M. Farnier and J. Davignon, *Current and future treatment of hyperlipidemia: the role of statins*, Am. J. Cardiol. 82(4B):3J-10J [1998]). Statins used in secondary
25 prevention of coronary artery or heart disease significantly reduce the risk of stroke, total mortality and
morbidity and attacks of myocardial ischemia; the use of statins is also associated with improvements in
endothelial and fibrinolytic functions and decreased platelet thrombus formation. (M. Kornitzer [1998];
M. Farnier and J. Davignon, *Current and future treatment of hyperlipidemia: the role of statins*, Am. J. Cardiol. 82(4B):3J-10J [1998]).

30 The use of statin drugs has recently decreased the need for surgical coronary revascularization,
known as coronary artery bypass graft (CABG). (B.M. Ritskind, *Clinical trials of reducing low-density
lipoprotein concentrations*, Endocrinol. Metab. Clin. North Am. 27(3):585-95, viii-ix [1998]). But
CABG is still a common surgical intervention for patients who develop atherosclerotic occlusion in
coronary arteries. Approximately 12,000-14,000 CABG procedures are performed annually. (G.F. Neitzel
35 et al., *Atherosclerosis in Aortocoronary Bypass Grafts*, Atherosclerosis 6(6):594-600 [1986]). The

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patient's own saphenous vein, or brachial or mammary artery, is used to bypass the affected coronary artery. The majority of CABG patients experience good long-term results, but 30-40% require a second CABG within 10-12 years after surgery, and continuing atherosclerosis in the graft is an important factor in late graft failure. (L. Campeau *et al.*, *The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in saphenous-vein coronary-artery bypass grafts*, N. Eng. J. Med. 336(3):153-62 [1997]).

5 Atherosclerosis in bypass grafts is associated with elevated serum levels of very low density lipoproteins (VLDL), low density lipoprotein cholesterol (LDL-C), and triglycerides, and low levels of high density lipoprotein cholesterol (HDL-C). (J.T. Lie *et al.*, *Aortocoronary bypass saphenous vein*

10 *atherosclerosis: Anatomic study of 99 vein grafts from normal and hyperlipoproteinemic patients up to 75 months postoperatively*, Am. J. Cardiol. 40:906 [1977]; L. Campeau *et al.*, *The relation of risk factors to the development of atherosclerosis in saphenous vein bypass grafts and the progression of disease in the native circulation*, N. Eng. J. Med. 311(21):1329-32 [1984]). It is standard for CABG

patients to be prescribed statin drugs to lower their serum LDL-C.

15 Lipid lowering therapy has been demonstrated to delay the progression of atherosclerosis in coronary arteries. (E.g., G. Brown *et al.*, *Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B*, N. Engl. J. Med. 323:1289-98 [1990]; J.P. Kane *et al.*, *Regression of coronary atherosclerosis during treatment of familial hypercholesterolemia with combined drug regimens*, JAMA 264:3007-12 [1990]; Jukema *et al.*, 1995).

20 Prior to the Post-CABG Trial, few data were available to determine the efficacy of LDL-lowering therapy to delay the obstruction of saphenous-vein grafts. (D.H. Blankenhorn *et al.*, *Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts*, JAMA 257:3233-40 [1987]). Furthermore, thrombosis had also been observed to contribute to graft obstruction

25 (G.F. Neitzel *et al.*, *Atherosclerosis in aortocoronary bypass grafts: morphologic study and risk factor analysis 6 to 12 years after surgery*, Arteriosclerosis 6:594-600 [1986]). Low-dose anticoagulation therapy prevented emboli after major surgery (A.G.G. Turpie *et al.*, *Randomised comparison of two intensities of oral anticoagulant therapy after tissue heart valve replacement*, Lancet 1:1242-45 [1988]; L. Poller *et al.*, *Fixed minidose warfarin: a new approach to prophylaxis against venous thrombosis after major surgery*, Br. Med. J. 295:1309-12 [1987]), and this implied that low-dose anticoagulation treatment would also be able to delay graft obstruction.

30 Statin drug treatment beneficially affects the long-term outcome for most CABG patients. In a large clinical study, the Post-CABG Trial, CABG patients received statin drug treatment to lower serum LDL-C; in comparing patients who had received aggressive lovastatin treatment (LDL-C lowered to 93-97 mg/dl) to those who had only received moderate lovastatin treatment (LDL-C lowered to 132-136 mg/dl), the percentages of patients with atherosclerotic worsening of grafts within 4 years were 39% and 51%, respectively, (L. Campeau *et al.* [1997]). The number of patients in the aggressive lovastatin-treatment group who required a second CABG procedure was 29% lower than the number in the moderate-treatment

group.

In addition to serum lipid concentrations, there are other risk factors, that may have a genetic basis, and that may independently affect atherosclerotic coronary artery disease and occlusion of bypass grafts or that interact with statin treatment to lower serum lipids, which can affect atherosclerotic stenosis.

5 Several laboratories have observed a link between variant alleles of the lipoprotein lipase gene (*LPL*) and the occurrence and/or progression of atherosclerosis. The involvement of *LPL* in coronary artery disease was suspected, since rare homozygotes for defects in this gene have type I hyperlipoproteinemia (OMIM 238600) and premature coronary artery disease. (P. Benlian *et al.*, *Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene*, *N. Engl. J. Med.* 10 335:848-54 [1996]).

Lipoprotein lipase (LPL; E.C. 3.1.1.34), also known as triacylglycerol acylhydrolase, is a heparin-releasable glycoprotein enzyme bound to glycosaminoglycan components of macrophages and to the luminal surface of capillary epithelial cells in a variety of human tissues, including heart, skeletal muscle, adipose, lung, and brain. (K.L. Wion *et al.*, *Human lipoprotein lipase complementary DNA sequence*, *Science* 235:1638 [1987]; C. Heizmann *et al.*, *DNA polymorphism haplotypes of the human lipoprotein lipase gene: possible association with high density lipoprotein levels*, *Hum. Genet.* 86:578-84 [1991]).

15 Lipoprotein lipase is active as a dimer of identical subunits, each approximately 62,500 D in unglycosylated form. (M.R. Taskinen *et al.*, *Enzymes involved in triglyceride hydrolysis*. In: James Shepard (Ed.), *Bailliere's Clinical Endocrinology and Metabolism*, Vol. 1, No.3, Bailliere Tindall, London, pp.639-66 [1987]).

20 Lipoprotein lipase is the rate-limiting enzyme for the hydrolysis and removal of triglyceride-rich lipoproteins, such as chylomicrons, VLDL, and LDL-C from the blood stream. (Jukema *et al.*, *The Asp₉ Mutation in the Lipoprotein Lipase Gene Is Associated With Increased Progression of Coronary Atherosclerosis*, *Circulation* 94(8):1913-18 [1996]). The enzymatic action of LPL results in the generation of mono- and diglycerides and free fatty acids that can be used as fuel for energy or reesterified for storage in peripheral adipose tissue.

25 The gene sequence of human *LPL* is known, including the 3' region through exon 10 and the 3' untranslated region (3'-UTR). (K.L. Wion *et al.*, *Human lipoprotein lipase complementary DNA sequence*, *Science* 235:1638-41 [1987]; T.G. Kirchgessner *et al.*, *The sequence of cDNA encoding lipoprotein lipase*, *J. Biol. Chem.* 262(18):8463-66 [1987]; K. Oka *et al.*, *Structure and polymorphic map of human lipoprotein lipase gene*, *Biochim. Biophys. Acta* 1049:21-26 [1990]; D. A. Nickerson *et al.*, *DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene*, *Nat. Genet.* 19:233-40 [1998]). Nickerson *et al.* sequenced the region of the *LPL* gene spanning exons 4-9 (containing the major catalytic portion of the enzyme) of 71 individuals taken from 3 different populations and observed 88 different DNA variants or polymorphisms, with 78 of these present at an allele frequency greater than 1% (D.A. Nickerson *et al.*, [1998]).

Two *LPL* polymorphisms are known to affect LPL activity. The D9N mutation in exon 2 has been

associated with increased triglyceride levels and with the occurrence of coronary atherosclerosis, attenuating the ability of pravastatin to lower LDL-C. (J. Jukema *et al.* [1996]). The N291S mutation in exon 6 has been associated with reduced HDL-C levels. (P. Reymer *et al.*, *A lipoprotein lipase mutation [asn291ser] is associated with reduced HDL cholesterol levels in premature atherosclerosis*, Nat. Gen. 5 10:28-34 [1995]; H.H. Wittrup *et al.*, *A common substitution [asn291ser] in lipoprotein lipase is associated with increased risk of ischemic heart disease*, J. Clin. Inves. 99:1606-13 [1997]). The N291S mutation is also linked with increased coronary stenosis (narrowing of arterial lumen) seen on angiography in women with verified ischemic heart disease compared to controls. (H.H. Wittrup *et al.* [1997]).

Two other *LPL* polymorphisms have demonstrated association with the development of atherosclerosis, although their functional significance is unknown. The first is the *PvuII* polymorphism in intron 6, which is linked with the number of coronary blood vessels with greater than 50% obstruction (X. Wang *et al.*, *Common DNA polymorphisms at the lipoprotein lipase gene: association with severity of coronary artery disease and diabetes*, Circulation 93:1339-45 [1996]). The second is the *HindIII* polymorphism in intron 8, associated with the angiographic severity of coronary artery disease. (R. Mattu

15 *et al.*, *DNA variants at the LPL gene locus associate with angiographically defined severity of atherosclerosis and serum lipoprotein levels in a Welsh population*, Arterio. Thromb. 14:1090-97 [1994]; R. Peacock *et al.*, *Associations between lipoprotein lipase, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden*, Atherosclerosis 97:171-85 [1992]).

20 Progress in pharmacogenetics has shown that human genetic variation underlies different individual responses to drug treatment within a population. (Reviewed in G. Alvan, *Genetic polymorphisms in drug metabolism*, J. Int. Med. 231:571-73 [1992]; P.W. Kleyn and E.S. Vesell, *Genetic variation as a guide to drug development*, Science 281:1820-22 [1998]). For example, alleles of the *NAT1* and *NAT2* genes (N-Acetyltransferases) create a “slow acetylator” phenotype in 40-60% of

25 Caucasians, resulting in a slow clearance and associated toxicity of many drugs including isoniazid and procainamide (K.P. Vatsis *et al.*, *Diverse point mutations in the human gene for polymorphic N-acetyltransferase*, Proc. Natl. Acad. Sci. USA 88(14):6333-37 [1991]). A defect in *CYP2D6* (a member of the cytochrome P450 family) leads to the “poor metabolizer” phenotype in 5-10% of Caucasians, affecting the metabolism of many drugs including some beta-blockers and antiarrhythmics. (Reviewed

30 in A.K. Daly *et al.*, *Metabolic polymorphisms*, Pharmac. Ther. 57:129-60 [1993]). Some genetic variation can be associated with the accumulation of toxic products, for example treatment of *TPMT*-deficient (thiopurine methyltransferase) patients with 6-mercaptopurine or azathioprine can lead to a potentially fatal hematopoietic toxicity due to higher than normal levels of thioguanine nucleotides. (R. Weinshilboum, *Methyltransferase pharmacogenetics*, Pharmac. Ther. 43:77-90 [1989]; E.S. Vesell, *Therapeutic lessons from pharmacogenetics*, Ann. Intern. Med. 126:653-55 [1997]).

The presence of multiple genetic and environmental factors capable of creating such large variations in how drugs operate in the patient argues that individualization of the choice of drug and dosage

is required for optimal treatment of disease, including atherosclerotic coronary artery disease. Jukema *et al.* (1996) reported that the HMG-CoA reductase inhibitor pravastatin did not lower the LDL-cholesterol level in subjects with the *LPL* N9 polymorphism to the same extent as in those with the *LPL* D9 polymorphism. In addition, J. A. Kuivenhoven *et al.* (1998) observed that pravastatin slowed the progression of atherosclerosis in subjects with the *CETP* B1B1 genotype, but not in those with the *CETP* B2B2 genotype. (J.A. Kuivenhoven *et al.*, *The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis*, N. Engl. J. Med. 338:86-93 [1998]). These reports suggest that there are interactions between statin drugs and some genetic determinants of atherosclerosis.

There has been a definite need for a reliable predictive test for determining which patients suffering from coronary artery disease, or which CABG patients, will likely not respond positively to statin drug treatment with respect to stenosis of a coronary artery or bypass graft. Such a genetic testing method can provide useful information so that patients can be given more individually suited alternative treatments to prevent further injury.

This and other benefits of the present invention are described herein.

SUMMARY OF THE INVENTION

The present invention relates to a method of detecting a genetic predisposition in a human subject for non-responsiveness to statin drug treatment for coronary artery disease. This genetic testing method involves analyzing amplification products of the nucleic acids in a human tissue sample that includes a non-coding or untranslated region within the 3' end of the human *LPL* gene. Homozygosity for a variant allele in a non-coding or untranslated region within the 3' end of the human *LPL* gene indicates a genetic predisposition for non-responsiveness to treatment with statin-class drugs, such as lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin, which are typically prescribed to treat atherosclerotic stenosis in subjects with coronary artery disease, or to prevent graft worsening (stenosis) in CABG patients.

The present invention also relates to oligonucleotide primer sequences, primer sets, and genetic testing kits for practicing the method.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows graft worsening in subjects related to different *LPL* variant alleles in the *LPL* gene.

Figure 1(a) shows the location of some variant alleles in the *LPL* gene. Vertical bars represent exons. Figure 1(b) shows the percentage of subjects with graft worsening. Each pair of vertical bars represents two genotype groups for each marker as defined in the box at the base of the bar. The number of subjects in each genotype group (N) is given below each bar. Figure 1(c) represents the odds ratios and 95% confidence limits for graft worsening for each polymorphism.

Figure 2 shows graft worsening in subjects by *Hind*III genotype and drug treatment groups. The

total number of subjects in each group is given on each vertical bar.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is directed to a method of detecting a genetic predisposition in a human subject for non-responsiveness to statin drug treatment for coronary artery disease or high blood pressure.

5 This genetic testing method involves analyzing amplification products of the nucleic acids in a human tissue sample for homozygosity with respect to a variant allele in a non-coding or untranslated region of the 3' end of the human *LPL* gene. The present invention does not rely on and is not committed to any particular mechanism by which a variant allele or *LPL* polymorphism in a non-coding or untranslated region of the 3' end of the human *LPL* gene produces a phenotype of non-responsiveness to statin drug

10 treatment.

The LPL gene is located on the short arm of human chromosome 8, at 8p22. (R.S. Sparkes *et al.*, *Human genes involved in lipolysis of plasma lipoproteins: Mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21*, *Genomics* 1:138-44 [1987]). The gene is near microsatellite marker D8S1715 and flanked by microsatellites D8S261 and D8S280. Closer flanking sequences of human LPL 15 are defined by GenBank accession numbers M94221 and M94222 (S. Wood *et al.*, *Support for founder effect for two lipoprotein lipase [LPL] gene mutations in French Canadians by analysis of GT microsatellites flanking the LPL gene*, unpublished [1992]). The gene spans about 30 kb and contains 10 exons encoding a 475 amino acid protein including a 27 amino acid secretory signal peptide. (S. Deeb and R. Peng, *Structure of the human lipoprotein lipase gene*, *Biochemistry* 28(10):4131-35 [1989]; T.G. 20 Kirchgessner *et al.*, *Organization of the human lipoprotein lipase gene and evolution of the lipase gene family*, *Proc. Natl. Acad. Sci. USA* 86:9647-51 [1989]).

The 3' end of the human lipoprotein lipase gene, for purposes of the present invention, includes nucleotide positions 4801 through 9734 of the Nickerson reference sequence extending from intron 6 into intron 9. (GenBank accession No. AF050163). (D. A. Nickerson *et al.*, *DNA sequence diversity in a 9.7-kb region of the human lipoprotein lipase gene*, *Nat. Genet.* 19:233-40 [1998]). The complete Nickerson reference sequence is the following:

1 TGTAACACAA AATTAAAATA AGTAGAATTAA GTTTCTAGTA TTTCTTATAT TTGGAAAAACAA
 61 ATATTTATAT TCATTTTGTG TTCTTTAGIT TTATTTTGG CAGAACTGTAA AGCACCTTCA
 121 TTTTCTTTTCTT CTTCCAAAAGG AGGAGTTAA CTACCCCTCTG GACAATGTCCT ATCTCTTGGG
 181 ATACAGCCCTT GGAGCCCCATG CTGCTGGCAT TGCAAGGAAGT CTGACCAATA AGAAAGTCAA
 241 CAGAAATTACT GTAAAGAAAG CAATTCTGGT GGTCTTATCA TAAGAGGTGA AAAGACTGTC
 301 ATTCCTGAGAG AGAATCAGAA CAATTCTGGT TAATACCCA CATGTGTGGT GTTCTCTCCG
 361 GAGACATGAC CAGCACTTGA TTATCTCAATT GTAGGGCTCTT TTATTAAGGGA TAAGAAAAAA
 421 CACAGACGCT CTCACTGGCT TACTATCCAC TGGCAATAGC ACAGAAATAA AGCATAATTAA
 481 CACACAAATGC CTGCAAGATT CTCTGGGAAG CCTTGTTCCT CCCACTCTCA GCTCTGTGTT
 541 TTAGTAGTGT AATGTCACAT CAGTACTAGG AGAAAAGAAG AAGGGCCCAT TCCAGAGGCC
 601 ACTTCGAAAG AAGACCGCTCA TCTAGGCAAAG GGTGTGGCAT ACACACAGAG AGAAAGAACCC
 661 CACCACTGTGTT TATACATCTT CTGCACTATCA TGCAAGAAATAA TCTACAAAGG GAAATCCAGC
 721 CATCCTGAGT GGAAATTGCT GCATAAGGAT AGTAAAGAG ACTCAAAATTC ATTCTAGAAG

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781 GAGCCAAGCC TCCCTTATG TCTCTCTAAG TAAAGATACC ATGACTGTAG ATAGGAGCT
 841 ATAAGAACATC TAAATAGCTG CCAGTGCATT CAATGATGA GCAGTGACAT GCGATGTC
 901 TAGCAATGGA AATTACAAA TCTGTGTTCC TGCTTTTTC CTTTTAAGG CCTCGATCCA
 961 GCTGGACCTA ACTTGTGAGTA TGCGAGAGCC CGAGTCGTC TTTCCTCTGA TGATGAGAT
 5 1021 TTGAGG TCTTACACAG CATTACAGCA GGGTCCCCG GTGGAAGCAT TGGATTCAG
 1081 AAACAGTTG GGCATGTTG CATTACCCG AATGGAGGTA CTTTCAGCC AGGATGTAAC
 1141 ATTGGAGAAG CTATCCGCTG GATTGAGAG AGAGGACTT GAGGTAATA TTATTGAGA
 1201 GCGAATTAA TGTTGACTCTT ATCCCTAAC CTTATTGACCA CAATGCTTA CTCAGTAGCT
 1261 TCAAAAGTATG TAGTTGATCAT ATACACATTG GCGCAAAATA TGTTCTGAA GAATTCTGCA
 1321 ATGTTGAGCA TGACCCATT AGAGCAGGC AGACAGCCAT TTTATCTTTT ATTACTACT
 1381 CTGAGGCTA CACTGAGGAG TGCACTTACA GTAGCAAGAG AAAAGGTTG GATTITAGAC
 1441 AGGAAGACTC CACTGACCTC ATAATGGCA TCATAAAATG CTATCTGCC ACATGTTGTC
 1501 ATACCTGAA TGACTCTCA AGGCCAATGG AAAGATTTTA GATGTTACTG GACAGAAGA
 1561 TGTTAATTG CATAAAATCTT CAAAATGTT GCAACATTAATGTTGTTTGTT TGAGATAG
 1621 TTAAATAGT TAGCTTGTTGTT TAAATTTGTTT ATTCTGTTT GTTTGTGTT TGAGATAG
 1681 TCTTATTCTA TTGCCCCAAGC TGGGGTGCAG TCACAACTC ACAGGGACTT GCAATGTTG
 1741 CCAGGCTGGT CTCAAACATCC TGGCCTCAAG TGATCTCTT GCCTCAGCT CCCAAAGTT
 1801 TGGGATTGCA GCTGTGACCC ACCAGGCCA GTTACGATT TATTTTTAAG AGCCCCTTG
 1861 ATACCTTATA GCAATTGGGA CCTACCTAGG ATTACCTCGT TATTTTTGTC CACGTAATAG
 1921 AACTTGTAGG ATATTGTTAC TATTGTTGAT TCTCTAAAAA ACTTACAAGG ATTCTATCT
 1981 TATGGCATG CTGATTATTT CTATGTCAT TTGATATAAA AGAGTGTAG TAGGGGAGA
 2041 ACCCTCAATT GTACATAATA TCAATGATAA AATACAATT ATTAAACAT TACCTCTTA
 2101 AGATGTTGGT CTGAAATAA CAAATGTC CTAACCTACA GTTTTCCAACT TTACACATTG
 2161 GGCTTAACAA CCATTGTTAG TTGAGAAGCA CGTGTGTTTG TGACTTAAAAA CTTTGAC
 2221 TTATGATGGG TTGGGGGGTT ATTAAAGTC TTGTTGTTTA CAGTTTTTT GACTTATGAA
 2281 GAAATTATG TAAGGCAAGG GGCAAGGTAGA TTGTTCTAGA AGCACCTAGA AGTGTGAGAC
 2341 ACTTTCAATG TAAGGAGAAG ATGAGATAAA CAAGGAAATC ACACCTCCAC CTGGAGGCT
 2401 TATTACAGCT TCATAAAACAT ACTTACAATAA TAAAGAAGCA CAAAGTCAA AAATCCCTG
 2461 TGAACTGCA ATCTTCACCTC TCTTGAAAGT GGGGGCCG CTACCAACAA GAATATCTCC
 2521 TGAAAATAGGG CCTACAACTCA TAAATGCACA GGACTATATC CTGGGGTGT TCTACTCTAA
 2581 CACCACATCT CACCTTAATTG AGACATGCCA AATGAAACAC TCTTGTGAA TTCTGCGGA
 2641 GATACAATCT TTGTTGTTCTT TTCTTACCA GTGTTGACC AGCTAGTGGAA GTGCTCCAC
 2701 GAGGCTCCA TTCACTCTC TATGCACTC TTGTTGAAATG AAGAAATCTC AAGTAAGGCC
 2761 TACAGGTGCA GTTCCAAAGA AGCCTTGTAG AAGGGCTCT GTTGTGAGTT TAGAAAAGAC
 2821 CGCTGCAACA ATCTGGGCTA TGAGATCAAT AAAGTCAGAG CCAAAAGAG CAGCAAAATG
 2881 TACCTGAGA CGCTGCTCTCA GATGCCCTAC AAAGGTAGGC TGGAGACTGT TGTAATAAG
 2941 GAAACCAAGG ATGCTCTATTG CTCATGCTC ATCTGATCAC ATGTACTGAT TCTGTCATT
 3001 GGAACAGAGA TGATGACTGG TTGTTCTAA CCTCTGAGCCC TTGTTGTTCT GTGATGAGG
 3061 GTTGTGATGG ATTCATTTG CTGAGGCTTC TAATTCCTA TGTCACCAAG GTCCAGTGC
 40 3121 TCACTGTGGG ATTCGACCC TTGCTCGCTG CCCTCCCCCTG TAATGTGGC CATTAGCATG
 3181 GGCTAGGCTA TCAAGCAGA GCTCAGAGCT CATTGGAAC CATCCACCTC GGGTCACAA
 3241 ATATAAACCC TTGTTGCAAATC TTCAACCTAC TTCTGCTTT TGTAATAATG TTTTTTTAAA
 3301 TTTCAGGTGAGT CAGGGGTAGG TAGTGTAGGT TGCTAAAAAG GTAAACCTTG GACATGGAG
 3361 TTTGTTGTCC AGATAATTC ATCACCCAGG TATTAAGCTT AGTACCCATT AGTACCTT
 3421 CCTGAAGCTC TCCCTCCCTCC CACCCCTCTG GAGGGCCAG TGTCTGTTG TCCCCCTCAT
 3481 GTGCTCATGCA AAAGTTTATG TAGGACAGG CCACACATC TCAATTACCAT ATTGTCAAG
 3541 GCTGGTTCA TGCCACCATCA AGCAAGTTGA TAGCCCCAG AGCCTAAAT ATTACTCC
 3601 TGCCCCCTTA CAGAATGTC ACACATTACA TAAAGGCAAG GACCATCTG CTTATTTT
 3661 TATTTATTTA ATTGAGATG AAGTCTAGCT TTCTCTAGG CTGGAGGAGA GGGGCATGAT
 3721 CTGGCTCAC CACAACCTCT GCCTCCCCGGG TTCAATGAT TCCCCTGCC CAGCCCTCCG
 3781 AGTAGCTGGG ATAACAGGCA TGACCATCA TGCCAGCTA ATTTTTGTTAT TTGAGATA

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3841 GAGGGGGTTT CACCGTGTG ACCAGGCTGG TCTCGAACTG CTGACCTAG GTGATCTGG
 3901 CTCCCTGGCC TCATCTGTCT TTTAAATGC AACTATTCTT GGAAAGGCAAG AATATCTCAC
 3961 ACCTCTAAG ATACTGCCAT TTGCCAGGA GTTGTGTTCA CACTTGAATT TCAAGCTTGG
 4021 CCTCTTGTGTT AGAGGGCAGC CAAAGGAAAT GTCTGGAAAA TGAGAGAGGA GGTCTTCCGA
 4081 TAAAGCGGT GAGGGGACAG AACTTCCAGGA AGGGTGGCTT TTGTTGAAATCAGATGAA
 4141 CCTGAGGGAA GGATGATAT TAAAGAACAG TGCCCCCAGG TAAAACATAT GGCAACCATG
 4201 TGTAAGGTGA TTCTTAGAAT CTGTAGAGGT GTCTTCTGTG GTATAGAGGT TGAGGCACCT
 4261 GTCTCTCAAG GAAACCTTAA CTCTTCAAA TCAGGCAATG CGTATGAGGT AAAGAGAGGA
 4321 CTGTTGGGAA ATATCTTGA AGACACAGAT AGGCTTCACT CATCCCTGCC TCTCTCCACCA
 4381 GTGGGGTCAA GGCTCTGTCA GTGCTCCCTA GGGGCACCTC ACCACTCCCA GCTCTTCTCG
 4441 CTCTGGCTG TCTCTGTCC TCGAAGGGT TTGCTTAATT CTCAATTCAA TGTCTCTTCA
 4501 TCTTTAGTA GCTGTGGGGT TTGTTGTTG TTCTCTGTG TTTGCTTAGT ATCTGACTAC
 4561 TTTTTAAATA TAAAAGAGA TGTATCTAA CAAATAGAG ATTTGTATCA GAAGTTCACA
 4621 ACATTTTAAATAAATTTT CACCTGGACA AGAGCTAAAGCAGCATAAATATGCTT
 4681 GCTATATTCTT AAACCATCAG TCTTAAGAGA TGTGTGTC AGCTTAAGAG AAATACATT
 4741 TAATAGACAT TAACACATAAAGAAGAAAATCAGGACAA GATAGTGGGA TATAGAAGAA
 4801 AAAACATTCC AAGAATTATT TTATTTATT ATTATTTAT TTATTTATT ATTATTTAT
 4861 TTGAGACCA CGGTTCTGCCT CAGTTACCCCA GGCTGGAGTC CAGCGGGCCTA ATCTTAACCT
 4921 ACTGCAACCT CTGCTTCTTCG GTTCAAGGCA GTGCTCTGCTT GAGTAACCTGG
 4981 GATTACAGG ACCGGCCACC AGCCGCAACT AATTCTGTAA TTTTCTTAG TAGAACACAGG
 5041 GTTTCACCAT GTTGGGCAAG CTAGTCTCAA ACTCTTGACC TGAGGTGATT CACCCACCAA
 5101 GGGCTCCCAA ATGTCGGGA TTACAGGCAT GAGGCCACCAT GCCTGGCCTC CAAAACCTCT
 5161 TTTTCTCCATCACTCATGG TTCTTATTTA GTCTCTGTG CTTTCTCTTT AACCTCTCC
 5221 CAGGGCCTTGGTCTGGGTGTTGGTAGA GACAGAGGA GGGGAGGGAGA GGAGATATAG
 5281 AAGTTCACACT ACCTGCTTCCC AGAGGCTGTC CTTAGTATAG AATACCTTAA GGGCTGGCTT
 5341 TACAAGGCAG TCCTCTGTGC CTCACTGTG GCTCAATGAA ATAAGTCTT TTTTAAAAA
 5401 AATTTTATTT ATTTCATAG GTTATTGGGG GAAACGGTGG TGTTGGTTA CATGAGTAAG
 5461 TTCTTGTAGA GTGATTGTG AGATTTGGT GTGGCAATT CCGAATGAA AAATCAACCGA
 5521 AATAAGTCTT ATGATGCCAC TACTAGACAC CTATCTGCA CTAGATGGTG GGGGAATTAA
 5581 GAGCATGGGC ATGATCTGTG CACCTGGAGCC CGCTTACAG TCAGGGTGGAA GGACAGACCT
 5641 ACTCATGAAA CAAACACAGT GACATATAGT GACACAGAAAG CAAATGCTAA ATATGCTTGC
 5701 TCCAGATGTC AAGGGCAAG TGCCAGG ATGGCGGAGT TCATGGAGAA AGCATCATGA
 5761 GTGTTGGCCTCTGTGTTT GATCTCTTCA GCACCCCTCA AGATGGCTA CTTCTCTAATG
 5821 CTGCTTGGCA ATTCAAGAC ATTGGGGTTT TTCTCTATGCA TATAACCACAA CTTCTCTGAA
 5881 AGGGAGTACA ATCAAGGTC TGCAATTCTT AGGTATGAC ACTGTCATG ATGAATCTT
 5941 TCCAAAGCCAC AACAGTGGT CCAAGTGTGTT GCACCTCCGG TTTGAGTGCT AGTGAGATAC
 6001 TTCTGTGGTT CTGAATTGCCC TCAACTATTG GGGTGTGAT ATTTCTATAA AGATTGATCA
 6061 ACATGTTGGCA ATTTCTCTCC CACRACTCTT CCATACCAA GTAAAGATTC ATTTCTCTGG
 6121 GACTGAGAGT GAAACCCATA CCAATCAGGC CTGGAGATT TCTCTGTATG GCACCTGGC
 6181 CGAGAGTGAG AACATCCCAT TCACCTGTG AGTAGCACAG GGGGGGGTC ATCATGGCAC
 6241 CAGTCCCTCC CTGCCCCATA CCCTTGCTGT GACGAGCAGA AGCAGAGAGC GATGCCCTAGA
 6301 AAACAAGTCTT TTGTTTAAATAATCAGAAAT TTCAAAATTG AGGTCTTCCC TCTTATTTGAT
 6361 ATTGAGAAAAA AATGCTTCA AATTGGCCAT TTATTTCTCA CTTACTAGTT ATATTTTTT
 6421 ATTATATCATC TTATATCTGT TTATTTCTT TATAAAGCTG CTGTTAACAA ATATAATTA
 6481 ACTATCTCAA AAGGTTTGAC ATTAAGAAAAA ATGAGCAATG GTAAACAGGA ACCACTCTAT
 6541 AGATGTACAT ATATATGTCA CAGAAATAAT AGTGTAGA AGTCCATGAA CAAAGTGTAA
 6601 GCCTTCTTTT TTCTTTTTTT TTGAGATGG AGTCTCTC CTATTTGCCCA
 6661 GGCTGGAGTC CAGTGTGTTG ATCTCAGCTG ACTGCAACCC CTACCTCCGG AGTTCAACAA
 6721 ATTCTCTGTG CTAGCCTCC CGAGTAGCTG GGGCTGCAGG TGCCCCACAC CATGCCAGC
 6781 TAATTTTGTG ATTTTTGTAGTA CGCAAGGGT CTACCAATGT TGCCCAAGCT GGTCTTGTGA
 6841 TCTGTATCTC AGGTGATCCA CCCGGCTCGG CCTCCCAAG TGCTGGGATT ACAGGTGTGA

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6901 GCCACCATGC CCAGCCTTACCTTACTACT AATCAAAGAA ATAAAAGTAA GGCAACTTGA
 6961 TACTTTTACA ATTACTAGAT GAACAAATCT TTAAAAAATAG CCAGTGCAGA CAAGGGTGTG
 7021 AAGCAGAACCA TGCGAACCTA CCATGCATCA TTACGGCTA GAACCTCCA GGTGCGGAAG
 7081 GTAGTATTCTT AATACTTTTC CATAGCTACA AAATATTATT ACATAGAAGG GAGTGATTT
 7141 TTATCTAAAGAATAGTACA AAACACATT TTAAAAAAACA TCAATTACAG
 7201 TCGTACCTAT ACTAGCTAA ATTAGAACCAGTACCA CATTGAGGCA GTGGGTTAAAT
 7261 GAATCGTGGT TTATCAAGTC ATTAAAATCA ATCTAGCCTT TAAACACTAT AATTGTAGA
 7321 AACCCAGGAA AACATAGTAA AAAATGGAAT ATAAAATCTA AAGAGARTAA AGAATAGAGA
 7381 ATCGTATGTG TGCTGATGTT GTAGCTAAAT AATGTTCAAG TATCACACAA AATTGAAAG
 7441 GAATACATGAA ATATGAAAT TTATTTCTG ATTGATTGAC TTTCAGGTTT TCTTTTAAAGA
 7501 TTGTATTAAGAATTTCTG CATTAGGATAA ATTTGCTGAA TGTGGATATA ATTTAAAATA
 7561 TACTAAATGC CTCGACCTT CATTGGAGT TCTTGTGAG ACATTTTGTG CATTTTTAA
 7621 AATATCCCTT AAATAATAAA GCTATTATAA TTGGAGAGG AGAAAAAAA GTGGGGGCA
 7681 GGGAGAGCTG ATCTCTATAA CTAAACCAAT TTATGCTTT TTGTTTAAAGG CTTGAGTTT
 7741 CCACAAATAA GACATACCTC TTCTAAATTAC ACACAGAGT AGATTTGGA GAACACTCTA
 7801 TGTGGAAGCT CAAATGGAG AGTGTGTTCACTTCTAGG GTCAAGCTGG TGGAGCAGTC
 7861 CCGGCTTCGC CTCAGAAG ATCAGAGTAA AAGCAGGAGA GACTCAGAAA AAGTAATTAA
 7921 ATGTTTTTC TTCTGACCTC TTCTGACCTT CAGGAGCTT CAGGACCTAG GGGCTGTATT
 7981 TCAGGNGCCT TCACACCTAC TTCTGACCC CAGGAGCTT CAGGAAACCT TTGTATTTTACTGTAGAT
 8041 GTAGATTTTC TTCTGAGCT TTCTTTTATC TTCTTATTTT TGGGGGGCGG GGGGGGAAGT
 8101 GACAGTATTT TTGTATTTCA TTGTAGGGAA ACATAAGCCC TGAATGCCTC ACAGTTTATC
 8161 AGTGAGAGCT GGGATTAGAA GTCAAGGAA TCAGCTTCTC ATTTGGCACT GTTCTTGTAA
 8221 AGTCAAAATAGTGGAGGAA CAAACCTCCG AGATGCTACC TGATAATCA AAGATTTCAA
 8281 CCAACCTTT CAAGAAGGGT GAGATCCTAA GATAATCTCA ACCTGCTCC GCAGCCCCAC
 8341 CCATGTGTCAC CCATAAAAATC AATTACACAG AGATGCTTAT AGGATTTAAAC GCTTTTATAC
 8401 TAAATGTGCT GGGATTGTC AAACATAGT GTGCTGTTAT TTGTATTTAA AAAAAACTCT
 8461 AAGTTAGGAT TGACAAATTA TTCTCTTTA TCATCTTGT TGATACACCA AAGAAGCAAA
 8521 CAAACAAACAA AAAAAAAAAGA GAAAAGATC TTGGGGATGG AATGTTTATA AAGAATCTT
 8581 TTTCACCTAG CAATGTCTAG CTGAAAGGCAG ATGGCCATAAT TCCTTAATGCA AGATGCTAAAG
 8641 AGATGGCAGA GTTGTATTTT TATCTCTCTG TTGTGAAGCC CCAGTAACT AAGACTGCTC
 8701 TAGGCTGTCT CGATGCCGTG CTATCTAAAT TAATCTAGCT GTGCTGTTAT TTGTATTTAA
 8761 GGCTCTCAAATTAACCTCTG ATCTGTGAT GGCTCTGAGTG TGACAGTTAA TTATGGGA
 8821 TATCAAAACAA ATACCCAGC ATGTCATGTT ATTATTTAA CAGTCCTGAC AGAACCTGTC
 8881 TTCTGGACAC AGTGTCTTGG ATTGTCTCTAC ATGGCCATAAT CACATCCATT TTCTTCCACA
 8941 GGGTGTCTT CTGTTCTAGG GAGAAAGTGT CTCTTGTGCA GAAAGGAAG GCACCTGCG
 9001 TATTTGTGAA ATGGCCATGAC AAGTCTCTGA ATAAGAAGTC AGGCTGGTGA GCATTCTGGG
 9061 CTAAAGCTG TGCGGCATCC TGACCTCTG TGACCTCTG CCCCCTAAGGGG GGCAAGCTTCA TGATCTCCTC
 9121 TTACCCCTAT CACCAAGCAGC TTGGCTCTGAC TCATGTCATCA AAAGCTTCA ATCACTCTT
 9181 CTAGTCCTT CTGCTATGTT ATCAATGGG TTGTGCTT TATGCTAACAT TTCTCTT
 9241 TTCTTCTCTC CTCTGTTTC TCCCTGGCCG GACCTTCAAC CCAGGCACAT ATTATGAGTT
 9301 TTATTTTACT CTTGGACTTA CCCTGTAATC TTCACTCTC CTTTTTTCTC TACTGCGTCT
 9361 CTGCTGACTT TGCAAGATGCC ATCTGCAGAG CATGTAACAC AAGTTAGTA GTGCGGTT
 9421 TTGGCTGGGG TGCACTCTT CCCAGGATGT ATTCAAGGGAA GTAAAGAGT CTCACTGCA
 9481 CACCTGCAGC CACATAGTTC TTGATCTCTC AAGTGCAGC ATACCTGGG ACACACAGC
 9541 AACAGGGCTG CCCCCAAGCAC CCATCTAAAC ACCCTCAAAG CTGCAAGCA AACAGAAATGA
 9601 GAGTTATAGG AACTCTCTT CTCTCTCTC TCCAAACAC TCTGTGCTCTC TTCTCTACCT
 9661 GACCTTCTAGG GCTATCTCAT TTGGCAGCTG TTAGCTGCA TTTTCCAGAG CGTCAGTACT
 9721 GAGAGGACAC TAAG (SEQ. ID. NO.: 80).

Also for purposes of the present invention, the 3' end of the human lipoprotein lipase gene

includes exon 10 and the 3' untranslated region (3'UTR), at least partially defined by nucleotide positions 1 through 3240 of the reference sequence of Oka *et al.*, (GenBank accession No. X52978 and X53518; K. Oka *et al.*, *Structure and polymorphic map of human lipoprotein lipase gene*, *Biochim. Biophys. Acta* 1049(1):21-26 [1990], Erratum: [Biochim Biophys Acta

5 1991 Nov 11;1090(3):357]). In the reference sequence of Oka *et al.*, the first and second polyadenylation signals are at nt. 15-20 and 411-416, respectively (in bold), and two analogous AGTAAA sequences are at nt. 468-473 and 529-534 (in bold). The poly(A) addition site is at nt. 439. The following is the reference sequence of Oka *et al.*:

10 1 GAATTCTCT TAAAAATAAA ATGATGTATG AITTTGTGTT GGCATCCCTT TTATTAATTC
 61 61 ATTTAAATTC TGGAATTGGG TTGTGACCCA GGGTGCATTA ACITTTAAAGA TTCACTAAAG
 121 121 CAGCACATAG CACTGGGAAC TCTGCTCCG AAAAACTTGT TTATATATAT CAAGGATGTT
 181 181 CTGGCTTAC ATTATTTTTA TTAGCTGTAA ATACATGTGT GGATGTGAA ATGGAGCTTG
 241 241 TACATATTGG AAAGGTATT GTGGCTATCT GCATTTATAA ATGTGTGGT CTAACGTAT
 301 301 GTGCTTATC CATGTATGGT CTACAGAGC CAACTCAGTC TTATGAAATG GGCTTAACA
 15 361 361 AAACAAAGAAA GAAACGTACT TAACCTGTG AAGAAATGGA ATCAGCTTTT **AAATAAAATTG**
 421 421 ACAACATTTT ATTACACAC TAAGTCATTA TTTTAAAGT **AAATTATTC**
 481 481 TTAGGTCAGA TTCACTCAGC ATATTTGAC TAAGTAACCA CTGACTTTG **TAAACCGAAG**
 541 541 AGCTTCTGAG AATTATAGT TACCGTATAG ATATTTTAA CATTATAATT GTATAAACG
 601 601 TAAAGAAAGC CTTACATATC CTTTAAACTG ACTATAGAAG AAAATGATAC AGAATTTCG
 20 661 661 CTGCTATAAG TACACAGGAC TATTCCTGCC TACACATATGC TTTTCACAA GCAAAATGTT
 721 721 AGACTAATAT AAGGCATCTT TGCCCATTTT ATAGTGTACA TCATCTCTAT TCTGAGGCC
 781 781 TCATTGTAG CTGIAACGCA AGTAGCATTTT GTGCAATAAA ATGAACTATT TGGGATGGGA
 841 841 GGGTACATCTT TTAGAACCTT TGCTTGGGT TGCTTGTATA ATTATAGCA TATAGTCAT
 901 901 TTATGCACTG AAGTAGGGAT TGCTTCTAG TACAGTCAGG AAGAATTAG CCCAGAAAAC
 25 961 961 AATTATTCAT ATGGCCACTG ACCCAAACCTT CCAGGGTGAAG GAGCAATGGC GTGATCATGG
 1021 1021 CTCACTGCAC CTCCACCTCC CAGGCTCAAG TGATTCCTCT GCCTCAGCTT CCAAGTAGA
 1081 1081 TGGTACTACA AGCACACGCC ACTGACACCA GCTAATTTC TTATTTTG TAGAGATGGG
 1141 1141 GGTTTCACTCA TGTTGCCAG GCTGGCTTA AATTCTGCC CTCAGTGTCTC TCCCCCTT
 1201 1201 GGCCTCCCAA AGTGTGGAA TTACAGGCAT GAGCCACCAT GTCCAGCTT GACCCAAACT
 30 1261 1261 TTATTTGCTA GTTACGTTATT GGGGGCTCTT GGAGTTGGG TCTCCCTGAG CAGGAGGGGG
 1321 1321 CTCCCCAGIT CACACTTGGC CACTGCCATT CAATTCCTGT TGATATGATC AACAGAGTAG
 1381 1381 ACAATTGCAATGTTGCTGA GGATGTGGAG AAGTGTGAAC CTGTGTAAGT GGCTGTGATGGG
 1441 1441 AATGTAAAAT GGCACAGCCA CTATGGGAA CAATTTGGTA GTATTTCCAAGTAAAGCAT
 1501 1501 AGAGTTAAC CCATATGACC CAGCAATTCC ACTCCTAGAT ATATACCCAA GAGAAATGAA
 35 1561 1561 AACACAGATC CACAAAGAT TGCACACACA GGTTCATAGC AGCATTAAATC AGATTAGTC
 1621 1621 CAAAGTGGAC ACCCAAATG TCCATGAATC TGTGAAAGAG ATAAGCAAAAT GTGACAAAT
 1681 1681 TCACATAATA AAATATTATT CAGAAGTAAAGAACAAGC AGCAGATATA TGATACAAACA
 1741 1741 CGATGGCCCT TGAAAAGCTT TAGCCATATG AAAGAAACCA GATGCAAAT GGAACCATGG
 1801 1801 CTTAGGGGAG GAGAACGGCA CAATGGTGTAAAGTGTGAG AGAGGAACAA AAAAGGCTTAC

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1861 TGCTCTGCTC CCAGGCCAAG TAACACAGGA GGAAAGAAAA TATCCACATA TGCAGGGCT
 1921 AAAGGAAGA GGTGTTCTCA AGCTGAAGCA GGAGGTTGGGA CTCAACTCTG GAGGTGGCC
 1981 TCACACACTG TACCAAATTG AGGACTAGCT AAAACAGGGA TGGGGGTGAA AGCACCTTT
 2041 CGTAAAGACAT GCCACCATT GTCCCGTCT CTCCTCTAA GGCCTTGCT TGCTCATGTC
 5 2101 AGCAAGCTTA TTGCCCCATCA TTCTCTCTAG TTACAGACAT CTGTGGAGCT CTGAGTTTT
 2161 TTGCTTAATCA TTTTTAGA ACCTGGTCTCA CTCTCTCTCC TTCTACACT AGTTCTGTC
 2221 TTATTATTAC TGATTTCAGT ACCCTCTGAGG TGATAGATT TATTTTCAA TGGCAGCCAC
 2281 AACACTACCT CCCATCTAT ATGTTCCCT GCAATGTGCT TTGACATCC CTATTAAGAG
 2341 TTGGAATCTA GTCACCCCCG TTTCTAGTC TCCCCACTCC TTGAACTTG TGTGGCCCT
 10 2401 AAAGATGCTT CTACTAGTAG AATAGAACTA AAATGACCTT GGACCAAGTGT GGGGTGCAAG
 2461 CCTTAACCTGG CCTGGCAGCT TCTGCTTGTG TTGCTCTGGG GCACTCACTC TTGGGAAACT
 2521 TCCCTTGGG ATCTCAGATT CATGATGCGG AAGTGTGAGC CACATGAAAA GAGCATATGG
 2581 TTGGTCTCTC AGCTCCAGC CAACAACCAAG TCTCGACTGT CAGCCATGTG AGTGGAGCAT
 2641 CTGGACCTC CGGCCAGTTG AGTGTCTAGA AGACTGCAGC TCGAGCTGGC ATCTGGATGC
 15 2701 AACCACTGA GAGACGCTCT GCCCCAGCCAA GCCCCAGCCAA CTCACAGTAC TATGAGAGAT
 2761 ACTAATACT TGTTGTTGTG TTGTTGTTGTT TATTTAACTT TAAGTTTTAG
 2821 CATACACGTT CACAAACGTC AGGTAGTTA CATAATGATA CCTGGGCCAT TTGGTGTG
 2881 TGACCCAGT AACTCGTCAT TTAACTATTAG GTATATCTCC AAATGCTATC CCTCCCCCT
 2941 CCCTAAGTTT TTAGGAGTTT GCTTTGCAAC GATAGATAGT TGAAACATCT GGATGATGCA
 20 3001 TCCAGTATTG TGCTCTCTCA CTGCCCTTAC CTCTCTCTC CCATGGCCCT GTCTCTAA
 3061 TCTACCTTCA CATAGAAACA TTCACTACAG TGCTATACCA TATCATGCCA TTACTAA
 3121 CTCTATAACT CAATTCAAC TTCTCCCTTC TTGACTACC ACATGCTATC TTTTACTTT
 3181 AATCAGTCTA GTCTCTCAG TTCAACAGCT CCTCAACTGC CCCAGGACCT CCAATACATT//
 (SEQ. ID.NO.:94).

25 Also for purposes of the present invention, the 3' end of the human lipoprotein lipase gene includes the intervening nucleotide sequence between the end of the Nickerson reference sequence in intron 9 and the beginning of the reference sequence of Oka *et al.*

A non-coding or untranslated region of the 3' end of the human *LPL* gene includes any non-transcribed or untranslated nucleotide sequence within the 3' end, including all intronic sequences. Included

30 are the part of intron 6 extending from Nickerson reference sequence position nt. 4801 through nt. 6086; intron 7 from nt. 6208 through nt. 7729; intron 8 from nt. 7913 through nt. 8941; and intron 9 from nt. 9047 through nt. 9734. Also included is exon 10 and the 3'UTR.

35 A variant allele in a non-coding or untranslated region of the 3' end of the human *LPL* gene is a mutation or polymorphism with respect to the Nickerson or Oka *et al.* reference sequences, of any class, such as, but not limited to, a single nucleotide polymorphism (SNP). Included among the sources of variant alleles in a non-coding or untranslated region of the 3' end of the human *LPL* gene are deletion mutations, insertion mutations, inversions, translocations, transitions, tranversions, or repeats.

Examples of homozygous genotypes that indicate a genetic predisposition to non-responsiveness

to statin drug treatment, in accordance with the present method, include, but are not limited to, the *Hind*III 2/2 and (TTTA)_n 4/4 genotypes.

The *Hind*III 2 variant allele, is created by a T to G transition in the single *Hind*III recognition site mapped in intron 8, i.e., AAGCTT to AAGCGT, at position 8393 of the Nickerson reference sequence.

5 (K. Oka *et al.* [1990]; C. Heinzmman *et al.*, *RFLP for the human lipoprotein lipase [LPL] gene: Hind*III, Nuc. Acids Res. 15:6763 [1987]; D. A. Nickerson *et al.* [1998]). For purposes of the present invention, nucleic acids comprising the normal locus of the *Hind*III recognition site in intron 8 of the human *LPL* gene are any nucleic acid sequences that overlap the entire six-basepair region at positions 8389-8394 of the Nickerson reference sequence, whether or not the nucleic acid sequence of a particular human subject at 10 that locus is AAGCTT.

The tetranucleotide (TTTA)_n repeat sequence in intron 6 of the *LPL* gene begins at position 4819 of the Nickerson reference sequence and extending to position 4864. There are five known (TTTA)_n alleles or polymorphisms. Allele 4 yields a 131 bp nucleotide fragment when PCR amplification is done using a primer set comprising reverse primer GZ-15 (5'-CCT GGG TAA CTG AGC GAG ACT GTG 15 TC-3'; SEQ. ID. NO.:33) and forward primer GZ-14 (5'- ATC TGA CCA AGG ATA GTG GGA TAT A-3'; SEQ. ID. NO.:34).

In the (TTTA)_n 4 variant allele, two additional TTTA repeats (shown below in underlined boldface type) are added to give the (TTTA)_n 4 allele length of 131 bp. Nucleotide position numbers with respect to the Nickerson reference sequence will be off from that point on:

20 4501 TCTTTAGTA GCTOTGGGT TTGTGTTG TCTCTCTGTT TTGCTTAGT ATCTGACTAC
 4561 TTTTAATTA TAAAAGAGA TGATCTAA CAAAATAGAG ATTTGTATCA GAAGTTCACA
 4621 ACATTTATA AAAATTTTTT CACCTGACCA AGAGCTAA GAGCATAAA AATATGGCT
 4681 GCTATATTCT AAACCATCAG TCTTAAAGA TCTGTGCTC ACCTTAAAG AAAATACATT
 4741 TAATGACAG TAACACAAAT AAGAAAAAAA TCTGACCAAO GATAGTGGGA TATAGAAGAA
 4801 AAAACATTC AACGATTATT TTATTTATTATTATT ATTATTATT TTATTATT ATTATTATT
 4861 TTTGAGACA CGGCTCTCGCT CAGTAAACCA GGCTGGAGTG CAGCGGCCA ATCTTAACCTC
 4921 ACTGCAACCT CTGCTTCCG GTTCAACCGA TTCTCTGCC TCAGCCTCT GAGTAACCTG
 4981 GATTACAGGC ACCGGCCACC ACGGCCAACT AATTTCGTGA TTTTCTTAG TAGAAACAGG
 5041 GTTTCACCAT GTTGGCCAACT CTAGTCTCAA ACTCCCTGACC TCAGGTGATT CACCCACCAA
 5101 GCGCTCCCAA AGTGCTGGG TTACAGGCAAT GAGGCCACAT GCGCTGGCTC CAAAACCTCT //
 (SEQ. ID. NO.:93).

A statin drug is any 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, including, but not limited to, lovastatin, pravastatin, simvastatin, atorvastatin, fluvastatin, and cerivastatin.

A human subject, particularly a CABG patient, who has a genetic predisposition for non-35 responsiveness to statin drug treatment possesses an hereditary inclination, susceptibility, or tendency to develop atherosclerotic stenosis of coronary blood vessels, including of a native coronary artery, or of any coronary artery bypass graft using a saphenous vein or any other vein or artery, in a manner that does not

respond to statin drug treatment. It does not mean that at any time such a person will actually develop stenosis of a coronary blood vessel, or graft worsening (graft lumen narrowing). It merely means that he or she has a greater likelihood of developing stenosis, when statin treatment is given; this is in comparison to the general population of individuals who are not homozygous for a mutation in the 3' end of the *LPL* gene, for example for the *HindIII* 2 allele or (TTTA)_n 4 allele, including those who have atherosclerotic coronary artery disease, who are coronary artery bypass graft patients.

5 A CABG patient is a human subject who is a candidate for coronary artery bypass graft surgery or one who has undergone a coronary artery bypass graft procedure.

10 Any human tissue containing nucleic acids can be sampled and collected for the purpose of practicing the methods of the present invention. A most preferred and convenient tissue for collecting is blood. Collecting a tissue sample includes in vitro harvest of cultured human cells derived from a subject's tissue or any means of in vivo sampling directly from a subject, for example, by blood draw, spinal tap, tissue smear or tissue biopsy. Optionally, tissue samples are stored before analysis by well known storage means that will preserve a sample's nucleic acids in an analyzable condition, such as quick freezing, or a 15 controlled freezing regime, in the presence of a cryoprotectant, for example, dimethyl sulfoxide (DMSO), glycerol, or propanediol-sucrose. Tissue samples can also be pooled before or after storage for purposes of amplifying them for analysis.

20 Amplifying nucleic acids from a tissue sample of a subject to obtain amplification products includes any conventional means of amassing sufficient nucleic acid material for analysis. Most preferably, amplification is by conventional polymerase chain reaction (PCR) methods. Alternatively, amplification of nucleic acids is by in vitro cell culture and harvest of the subject's cultured cells, or by multiple sampling from the subject's tissues in vivo and pooling of multiple tissue samples from a subject. Nucleic acids thus amplified are amplification products if they include a non-coding or untranslated nucleotide sequence from the 3' end of the *LPL* gene, for example, the normal locus of the *HindIII* recognition site in intron 8, 25 or the tetranucleotide (TTTA)_n repeat region of intron 6, of the human *LPL* gene.

20 In a preferred embodiment of the present method, nucleotide sequencing is used to analyze the amplification products of the nucleic acids in a tissue sample to detect homozygosity for a mutation in the 3' end of human *LPL*. The skilled artisan can detect the mutation by any nucleotide sequencing means, for example conventional dideoxy sequencing or preferably by using a commercially available automated sequencer, then comparing the subject's nucleotide sequences to other known human *LPL* sequences available in genomic sequence databases, such as GenBank.

30 In a most preferred embodiment that employs nucleotide sequencing, sequencing of 3' end *LPL* sequences is accomplished by using fluorescence-based single strand conformation polymorphism analysis (SSCP), a routine and reliable means of identifying point mutations, small insertions or deletions. (J.S.

35 Ellison, *Fluorescence-based mutation detection. Single-strand conformation polymorphism analysis [F-SSCP]*, *Mol. Biotechnol.* 5(1):17-31 [1996]; H. Iwahana *et al.*, *Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, *Biotechniques* 21(3):510-14, 516-19

[1996]; R. Makino *et al.*, *F-SSCP: fluorescence-based polymerase chain reaction-single-strand conformation polymorphism [PCR-SSCP]*, PCR Methods Appl. 2(1):10-13 [1992]). An automated system may be used, such as an Applied Biosystems DNA sequencer, equipped with GENESCAN 672®, Genotyper®, or another appropriate analytical software package.

5 Optionally, high throughput analysis may be achieved by PCR multiplexing techniques well known in the art. (E.g., Z. Lin *et al.*, *Multiplex genotype determination at a large number of gene loci*, Proc. Natl. Acad. Sci. USA 93(6):2582-87 [1996]).

In a most preferred embodiment, nucleotide sequencing is unnecessary for analyzing the amplification products. For example, heteroduplex analysis on high resolution gel matrices are employed

10 to detect even single nucleotide polymorphisms. (M.T. Hauser *et al.*, *Generation of co-dominant PCR-based markers by duplex analysis on high resolution gels*, Plant. J. 16(1):117-25 [1998]). The PCR/OLA procedure can be used for analyzing amplification products to detect SNPs in the 3' end of the human *LPL* gene. (B.R. Glick and J. J. Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press, Washington, D.C., pp. 197-200 [1994]). Conformation-sensitive gel

15 electrophoresis of amplification products may also be employed as a means of analysis by the skilled artisan in practicing the methods of the present invention. (A. Markoff *et al.*, *Comparison of conformation-sensitive gel electrophoresis and single strand conformation polymorphism analysis for detection of mutations in the BRCA1 gene using optimized conformation analysis protocols*, Eur. J. Genet. 6(2):145-50 [1998]).

20 Electrophoresis for analyzing amplification products is done rapidly and with high sensitivity by using any of various methods of conventional slab or capillary electrophoresis, with which the practitioner can optionally choose to employ any facilitating means of nucleic acid fragment detection, including, but not limited to, radionuclides, UV-absorbance or laser-induced fluorescence. (K. Keparnik *et al.*, *Fast detection of a (CA)18 microsatellite repeat in the IgE receptor gene by capillary electrophoresis with*

25 *laser-induced fluorescence detection*, Electrophoresis 19(2):249-55 [1998]; H. Inoue *et al.*, *Enhanced separation of DNA sequencing products by capillary electrophoresis using a stepwise gradient of electric field strength*, J. Chromatogr. A. 802(1):179-84 [1998]; N.J. Dovichi, *DNA sequencing by capillary electrophoresis*, Electrophoresis 18(12-13):2393-99 [1997]; H. Arakawa *et al.*, *Analysis of*

30 *single-strand conformation polymorphisms by capillary electrophoresis with laser induced fluorescence detection*, J. Pharm. Biomed. Anal. 15(9-10):1537-44 [1997]; Y. Baba, *Analysis of disease-causing genes and DNA-based drugs by capillary electrophoresis. Towards DNA diagnosis and gene therapy for human diseases*, J. Chromatogr B. Biomed. Appl. 687(2):271-302 [1996]; K.C. Chan *et al.*, *High-speed electrophoretic separation of DNA fragments using a short capillary*, J. Chromatogr B. Biomed. Sci. Appl. 695(1):13-15 [1997]). Any of diverse fluorescent dyes can optionally be used to label primers of

35 the present invention or amplification products for ease of analysis, including but not limited to, SYBR Green I, Y10-PRO-1, thiazole orange, Hex (i.e., 6-carboxy-2',4',7,4,7-hexachlorofluorescein), pico green, edans, fluorescein, FAM (i.e., 6-carboxyfluorescein), or TET (i.e., 4,7,2',7'-tetrachloro-6-

carboxyfluorescein). (E.g., J. Skeidsvoll and P.M. Ueland, *Analysis of double-stranded DNA by capillary electrophoresis with laser-induced fluorescence detection using the monomeric dye SYBR green I*, *Anal. Biochem.* 231(20):359-65 [1995]; H. Iwahana *et al.*, *Multiple fluorescence-based PCR-SSCP analysis using internal fluorescent labeling of PCR products*, *Biotechniques* 21(30):510-14, 516-19 [1996]).

5 Analyzing the amplification products can also be done by means of restricting the amplification products with one or more restriction enzymes. When the amplification products comprise the normal locus of the *Hind*III recognition site in intron 8 of the human *LPL* gene, the restriction enzyme employed is preferably *Hind*III. Restriction of nucleic acids is followed by separation of the resulting fragments and analysis of fragment length or differential fragment migration in denaturing high-performance liquid 10 chromatography (DHPLC) or gel electrophoresis, as above, including restriction-capillary electrophoresis. For example, this can be achieved by techniques known in the art, such as PCR-restriction fragment-SSCP, which can detect single base substitutions, deletions or insertions. (M. Tawata *et al.*, *A mass screening device of genome by polymerase chain reaction-restriction fragment-single strand conformation polymorphism analysis*, *Genet. Anal.* 12(3-4):125-27 [1996]; H.H. Lee *et al.*, *Mutational analysis by a 15 combined application of the multiple restriction fragment-single strand conformation polymorphism and the direct linear amplification DNA sequencing protocols*, *Anal. Biochem.* 205(2):289-93 [1992]).

The present invention also relates to an oligonucleotide primer for detecting a genetic predisposition for non-responsiveness to statin drug treatment in a human. Useful oligonucleotide primers for amplifying the nucleic acids include any 15 to 28-mer nucleotide sequence that hybridizes with a nucleic 20 acid fragment of the Nickerson or Oka reference sequences, under conventional conditions of stringency used for hybridization in PCR, and together in a set with another primer sequence amplifies a non-coding or untranslated region within the 3' end of the human *LPL* gene. A preferred primer is a 20 to 24-mer.

Useful for amplifying non-coding or untranslated nucleic acid sequences from intron 6 (beginning at position 5988 of the Nickerson reference sequence) through intron 9, is a set of oligonucleotide primers 25 having nucleotide sequences that are fragments of the nucleotide sequences in GenBank accession numbers M76722 (below) and M76723 (opposite strand). The nucleotide sequence of M76722 is the following:

1 GAATTCAAGG TCTGCATTT CTAGGTATGA ACACGTGCA TGATGAACTC TTTCCAAGCC
 61 ACACCAGTGG TTCCATGTG TGCACTTCC GGTGAGTG CTAGTGAGAT ACTTCTGTGG
 121 TCTCATGTT CCTGACTATT TGGGGTTGTG ATATTCAT AAAGATTGAT CAACATGTT
 181 GAAATTCCCT CCCAACAGTC TTCCATTACC AAGTAAGAT TCATTTTCTT GGGACTGAGA
 241 GTGAAACCCA TACCAATCG GCCTTGAGA TTCTCTGTA TGCCACCGTG GCCGAGAGTG
 301 AGAACATCCC ATTCACTCTG TGAGTAGCAC AGGGGGCGG TCATCATGGC ACCAGTCCT
 361 CTCCCTGCCAT AACCCCTGGT CTGAGCAGCA GAAGCAGAGA GCGATGCCA GAAAACAAGT
 421 CTTTAGTTAA AAAATCAGA ATTCAAAAT TGAGGTTCTT CCTCTATTG ATATGAGAA
 481 AAAATGCTT CAAATGGCC ATTTTATTCT CACTCTAG TTATTTTT TTATTTATCA
 541 TCTTATATCT GTTTATCTT TTATAAACG TGCTGTTAAA CAATATAATT AACTATCTC
 601 AAAAGGTTTG ACATTAAGA AAATGAGCAA TGGTAACAGG AAACCACTCT ATAGATGTAC
 661 ATATAATATG TACAGAAAAT ATAAGTAGTA AGAAGTCCAT GACAAAGTGT TAGCTCTTT

721 TTTTTTTT TTTTTTTT TTTTGAGAT GGAGTCTCTC TCTATTGCCG AGGCTGGAGT
 781 GCACTGATTC GATCTCAGCT CACTGCAACC TCTACCTCCC GAGTCAAC AATTCTCTG
 841 TCTCAGCCTC CCGAGTAGCT GGGGCTGCA GTGCCACCA CCATGCCAG CTAATTCTTG
 901 TATTTTAGT AGCAGACAGGG TCTCACCATG TTGGCCAAGC TGGCTCTGAA TTCTGTACT
 5 961 CAGGTGATCC ACCCGCTCG GCCTCCAAA GTGCTGGAT TACAGGTGTG AGCCACCATG
 1021 CCCAGCCTAC CTTTACTAC TAATCAAAGA AATAAAAGTA AGGCAACTTG ATACTTTAC
 1081 AATTACTAGA TGACAAATC TTAAAAAATA GCCAGTGCAG ACAAGGTGGT GAAGCAGAAC
 1141 ATGCGAACCTT ACCATGCATC ATTACCGCT AGAACCTCC AGGTGCGGAA GGTAGTATT
 1201 TATAACTTT CCATGCTAC AAAATATTAT TACATAGAAG GGAGTGTATT TTCTTAATA
 10 1261 TTATCTAA AGAAATAGTC AACAAACATT TTAAAAAACA TCAATTACAG TCGTACCTAT
 1321 ACTAGCATAA ATTAGAACCC CAGTATCCAA CATIGAGGCA GTGGGTAAT GAATCGTGGT
 1381 TTATCAAGTC ATTAAAATCA ATCTAGCCTT TAAAAACTAT AATITGAGGA AACCAGGAA
 1441 AACATAGTAA AAAATGGAAT ATAAAATCTG AAGAGAATAA AGAATAGAGA ATCGTATGT
 1501 TGCTATGATT GTAGCTAAAT AATGTCAGA TATCAACACA ATTGAAAAG GAATACATGA
 15 1561 AAATGAAAAT TATTTCTG AATGATTGAC TTCAAGGATTT TCTTTAGAA TTGTATTAA
 1621 TAGTTCATGT CATTAGGATA AATGCTGGAA TGTTGGATA ATTAAAATA TACTAAATGC
 1681 CTCGCAATT CATTGAGT TCTTGTGG ACATTTTGT GCATTTTAA AATATCCCT
 1741 AAAATAAAAA GCTATTATA TTGGAGAGG AGAAAAAAA GTGGGGGGCA GGAGAGCTG
 1801 ATCTCTATAA CTAAACCAAT TTATGCTTT TTGTTGAGG CCTGAAAGTT CCACAAATAA
 20 1861 GACCTACTCC TTCTTAATT ACACAGAGGT AGATATGGA GAACTACTCA TTGTTGAAGCT
 1921 CAAATGGAAG ATGATTATC ACITTAGCTG GTCAAGACTGG TGAGGACAGTC CCGGCTTCG
 1981 CATTCAAGG ATCAGAGTAA AAGCAGGAGA GACTCAGAAA AAGTAATTAA ATGTTTTT
 2041 CTTCCTTCAC TTAGACCCCC CACCTGATGT CAGGACCTAG GGGCTGTATT TCAGGGGCT
 2101 TCACAATTCA GGGAGAGCTT TAGGAAACCT TGTATTATT ACTGTATGAT GTAGATTTC
 25 2161 TTAGGAGTC TTCTTTATT TTCTTATT TGGGGGGGG GGGGGGAAGT GACAGTATT
 2221 TTGTTATTCA TGAGGAAA ACATAAGCCC TGAATGCTC ACAGTTATT AGTGGAGAGCT
 2281 GGATTAGAA GTCAAGGATC TCAGCTCTT ATTGCGACT GTTCTGTG AGTACAAAAT
 2341 AGTTAGGAA CAAACCTCG AGATGTCACC TGATAATCA AAGATCAAA CCAACCTCTT
 2401 CCAGAAGGGT GAGATTCAA GATAATCTCA ACCTGCTCC GCAGCCCCAC CCATGTCAC
 30 2461 CCATAAAATG ATTACACAG AGATGCTAT AGGATTAAAG GCTTTATAC TAAATGCT
 2521 GGATTTGC AAATATAGT GTGCTGTAT TGTTAATTAA AAAAAGCTCT AAGTTAGGAT
 2581 TGACAAATTAA TTCTCTTAA GTCAATTGCT TGATCACC AAGAAGCAAA CAAACAAACA
 2641 AAAAAGGAA GAAAAGATC TTGGGGATGG AAATGTTAA AAGAATCTT TTACACTAG
 2701 CAATGCTAG CTGAAAGGAG ATGCCCTAA TCTTAATGC AGATGCTAAG AGATGGCAGA
 35 2761 GTTGTACTT TATCATCTCT TGGTAAAGC CCAGTAAACAT AAGACTGCTC TAGGCTGCT
 2821 GCATGCCCTGCTCTATCAAT TAACTGCTT GGTGCTGAA CACCAGGTTA GGCTCTCAA
 2881 TTACCCCTGCT ATTCTGATGT GGCCCTGAGTG TGACAGTAA TTATGGGAA TATCAAAACA
 2941 ATTACCCAGC ATGATCATGT ATTATTAAA CAGTCTGAC AGAACTGTCAC CTTGTGAAAC
 3001 AGTGTCTTGT ATTGTCTAC ATGGCATATT CACATCATT TTCTTCCACA GGGTGTACTT
 40 3061 CTGTTCTAGG GAGAAAGTGT CTCATTGCA GAAAGGAAAG GCACCTGCGG TATTGTGAA
 3121 ATGCCATGAC AAGTCTCTGA ATAAGAAGTC AGGCTGGTGA GCATTCTGGG CTAAGCTGA
 3181 CTGGGCATCC TGACCTTGCAC CCCTAAAGGA GGCAGCTTCA TGCAATTCTC TTCACCCCAT
 3241 CACCAGCAGC TTGGCCCTGAC TCAATGATC AAAGCATTCA ATCAGTCTTT CTAGTCTT
 3301 CTGCTATGAT ATCAAATGGG TCTGTGCTT TATGCAATAC TTCTCTTT TTCTTCTC
 45 3361 CTCTGTGTTTCCCGACCCGACCTCAAC CCAGGCACAC ATTAGGTT TTCTTACT

3421 CCTTGAACTA CCCCTGAATC TTCACTTCTC CTTTTTCTC TACTGCGTCT CTGCTGACTT
 3481 TGCGAGATGCC ATTCGAGAG CATGTAACAC AAGTTAGTA GTTGCCTTC TGCGCTGGG
 3541 TGCGAGCTCTT CCCAGGATGT ATTCAAGGGAA GTAAAAAGAT CTCAGTCAT CACCTGAGC
 3601 CACATAGTTC TTGATTCTCC AAGTGCAGC ATACTCCGGG ACACACAGCC AACAGGGCTG
 3661 CCCAAGCAC CCATCTCAA AACCCCTAA GCTGCAAGC AAACAGAAATG AGAGTTAG
 3721 GAAACTGTTC TCTCTTCTAT CTCCAAACAA CTCTGTGCC CTTTCCTTAC TGACCTTAG
 3781 GGCTAATCCA TGTTGCAGCT GTTAGCTGCA TCTTTCCAGA GCGTCAGTAC TGAGAGGACA
 3841 CTAAGCATGT GACCTTCACT ACTCTGTTC TGAATT (SEQ. ID. NO.:81).

For example, oligonucleotide primer sequences that are useful for amplifying nucleic acids that

10 comprise the normal locus of the *Hind*III recognition site in *LPL* intron 8, include but are not limited to the following sequences (designation after the SEQ. ID. NO. includes the nucleotide position within M76722, e.g., 2701 or 2397, at which the 5'-terminus of the primer sequence begins if it is an upper [“U”, i.e., forward] primer; at which position complementary to a position within M76722 its 3'-terminus ends if it is a lower [“L”, i.e., reverse] primer; and the primer length, e.g., 24 bases):

15 5'-GCA TCT GCC TTC AGC TAG ACA TTG-3' (SEQ. ID. NO.:1; LPL HindIII:2701L24);
 5'-TCT TCC AGA AGG GTG AGA TTC CAA-3' (SEQ. ID. NO.:2; LPL HindIII:2397U24);
 5'-GGA AAA CAT AAG CCC TGA ATC-3' (SEQ. ID. NO.:3; LPL HindIII:2236U21);
 5'- GAA AAC ATA AGC CCT GAA TCG-3' (SEQ. ID. NO.:4; LPL HindIII:2237U21);
 5'-AAC ATA AGC CCT GAA TCG CTC-3' (SEQ. ID. NO.:5; LPL HindIII:2240U21);
 20 5'-CCT GAA TCG CTC ACA GTT ATT-3' (SEQ. ID. NO.:6; LPL HindIII:2249U21);
 5'-CTG AAT CGC TCA CAG TTA TTC-3' (SEQ. ID. NO.:7; LPL HindIII:2250U21);
 5'-AAT CGC TCA CAG TTA TTC AGT-3' (SEQ. ID. NO.:8; LPL HindIII:2253U21);
 5'-TTG GCA CTG TTT CTT GTA AGT-3' (SEQ. ID. NO.:9; LPL HindIII:2313U21);
 5'-CAC TAT AGT TTG CAA AAT CCC-3' (SEQ. ID. NO.:10; LPL HindIII:2521L21);
 25 5'-CAAACCTCC GAG ATG CTA CCT GGA-3' (SEQ. ID. NO.:11; LPL HindIII:2351U24);
 5'-AGATGCTACCTG GAT AAT CAA AGA-3' (SEQ. ID. NO.:12; LPL HindIII:2361U24);
 5'-GATGCTACC TGG ATA ATC AAA GAT-3' (SEQ. ID. NO.:13; LPL HindIII:2362U24);
 5'-CTTCCAGAA GGG TGA GAT TCC AAG-3' (SEQ. ID. NO.:14; LPL HindIII:2398U24);
 5'-CCAGAAGGGTGA GAT TCC AAG ATA-3' (SEQ. ID. NO.:15; LPL HindIII:2401U24);
 30 5'-CAGAAGGGTGAG ATT CCA AGA TAA-3' (SEQ. ID. NO.:16; LPL HindIII:2402U24);
 5'-CCCACCCAT GTG TAC CCA TAA AAT-3' (SEQ. ID. NO.:17; LPL HindIII:2446U24);
 5'-CCACCCATG TGT ACC CAT AAA ATG-3' (SEQ. ID. NO.:18; LPL HindIII:2447U24);
 5'-CCCATGTTG ACC CAT AAA ATG AAT-3' (SEQ. ID. NO.:19; LPL HindIII:2450U24);
 5'-GTACCCATA AAA TGA ATT ACA CAG-3' (SEQ. ID. NO.:20; LPL HindIII:2457U24);
 35 5'-CCCATAAAATGA ATT ACA CAG AGA-3' (SEQ. ID. NO.:21; LPL HindIII:2460U24);
 5'-ATGAATTAC ACA GAG ATC GCT ATA-3' (SEQ. ID. NO.:22; LPL HindIII:2468U24);
 5'-ACACAGAGA TCG CTA TAG GAT TTA-3' (SEQ. ID. NO.:23; LPL HindIII:2475U24);
 5'-TTATAA CAT TTC CAT CCC CAA GAT-3' (SEQ. ID. NO.:24; LPL HindIII:2658L24);
 5'-CATCTG CCT TCA GCT AGA CAT TGC-3' (SEQ. ID. NO.:25; LPL HindIII:2700L24);
 40 5'-CTGCAT TAA GGA ATT AGG GCA TCT-3' (SEQ. ID. NO.:26; LPL HindIII:2719L24);
 5'-AGATCA ACT CTG CCA TCT CTT AGC-3' (SEQ. ID. NO.:27; LPL HindIII:2745L24);

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5'-TCT TAT GTT ACT GGG CTT TCA CCA-3' (SEQ. ID. NO.:28; LPL HindIII:2781L24);
 5'-AGCCTA GAG CAG TCT TAT GTT ACT-3' (SEQ. ID. NO.:29; LPL HindIII:2793L24);
 5'-CAGCCT AGA GCA GTC TTA TGT TAC-3' (SEQ. ID. NO.:30; LPL HindIII:2794L24);
 5'-ACAGCC TAG AGC AGT CTT ATG TTA-3' (SEQ. ID. NO.:31; LPL HindIII:2795L24);
 5 5'-AGACAGCCT AGA GCA GTC TTA TGT-3' (SEQ. ID. NO.:32; LPL HindIII:2797L24);
 5'-CTTATAACATTTCCATCCCCAAG AT-3' (SEQ. ID. NO.:35; LPL HindIII:2658L26);
 5'-TGTACCCATAAAATGAATTACACAGA-3' (SEQ. ID. NO.:36; LPL HindIII:2456U26);
 5'-ACCCATAAAATGAATTACACAGAGAT-3' (SEQ. ID. NO.:37; LPL HindIII:2459U26);
 5'-AAAATGAATTACACAGAGATCGCTAT-3' (SEQ. ID. NO.:38; LPL HindIII:2465U26);
 10 5'-TTACACAGAGATCGCTATAGGATTAA-3' (SEQ. ID. NO.:39; LPL HindIII:2473U26);
 5'-CAGCCTAGAGCAGTCTTA TGT TAC T-3' (SEQ. ID. NO.:40; LPL HindIII:2793L25);
 5'-ACAGCCTAGAGCAGTCTTATG TTA C-3' (SEQ. ID. NO.:41; LPL HindIII:2794L25);
 5'-GACAGCCTAGAGCAGTCTTAT GTT A-3' (SEQ. ID. NO.:42; LPL HindIII:2795L25);
 5'-ATAAAATGAATTACACAGAGATCGCTAT-3' (SEQ. ID. NO.:43; LPL HindIII:2463U28);
 15 5'-AAGATTCTTATAACATTCATC CC-3' (SEQ. ID. NO.:44; LPL HindIII:2664L26);
 5'-AATTACACAGAGATCGCTATAGGATTAA-3' (SEQ. ID. NO.:45; LPL HindIII:2471U28);
 5'-ACAGCCTAGAGCAGTCTTATGTTACT-3' (SEQ. ID. NO.:46; LPL HindIII:2793L26);
 5'-CCC ACC CAT GTG TAC CCA T-3' (SEQ. ID. NO.:47; LPL HindIII:2446U19);
 5'-CCA CCC ATG TGT ACC CAT-3' (SEQ. ID. NO.:48; LPL HindIII:2447U18);
 20 5'-CAC CCA TGT GTA CCC ATA AAA-3' (SEQ. ID. NO.:49; LPL HindIII:2448U21);
 5'-ACC CAT GTG TAC CCA TAA AA-3' (SEQ. ID. NO.:50; LPL HindIII:2449U20);
 5'-GGC TTT CAC CAA GAG ATG ATA A-3' (SEQ. ID. NO.:51; LPL HindIII:2770L22);
 5'-GGG CTT TCA CCA AGA GAT GAT A-3' (SEQ. ID. NO.:52; LPL HindIII:2771L22);
 5'-TGA ATT ACA CAG AGA TCG CTA T-3' (SEQ. ID. NO.:53; LPL HindIII:2469U22);
 25 5'-ACA GAG ATC GCT ATA GGA TTT A-3' (SEQ. ID. NO.:54; LPL HindIII:2477U22);
 5'-GTT ACT GGG CTT TCA CC-3' (SEQ. ID. NO.:55; LPL HindIII:2782L17);
 5'-CTT ATG TTA CTG GGC TTT CA-3' (SEQ. ID. NO.:56; LPL HindIII:2784L20);
 5'-TCT TAT GTT ACT GGG CTT TC-3' (SEQ. ID. NO.:57; LPL HindIII:2785L20);
 5'-CCA CCC ATG TGT ACC CAT A-3' (SEQ. ID. NO.:58; LPL HindIII:2447U19);
 30 5'-CAC CCA TGT GTA CCC ATA-3' (SEQ. ID. NO.:59; LPL HindIII:2448U18);
 5'-ACC CAT GTG TAC CCA TAA-3' (SEQ. ID. NO.:60; LPL HindIII:2449U18);
 5'-CCC ATG TGT ACC CAT AAA-3' (SEQ. ID. NO.:61; LPL HindIII:2450U18);
 5'-CAA CTC TGC CAT CTC TTA GC-3' (SEQ. ID. NO.:62; LPL HindIII:2745L20);
 5'-TCA ACT CTG CCA TCT CTT AG-3' (SEQ. ID. NO.:63; LPL HindIII:2746L20);
 35 5'-ATC AAC TCT GCC ATC TCT TA-3' (SEQ. ID. NO.:64; LPL HindIII:2747L20);
 5'-GAA AAC ATA AGC CCT GAA-3' (SEQ. ID. NO.:65; LPL HindIII:2237U18);
 5'-AAA ACA TAA GCC CTG AAT C-3' (SEQ. ID. NO.:66; LPL HindIII:2238U19);
 5'-ACA TAA GCC CTG AAT CG-3' (SEQ. ID. NO.:67; LPL HindIII:2241U17);
 5'-CTG AAT CGC TCA CAG TT-3' (SEQ. ID. NO.:68; LPL HindIII:2250U17);
 40 5'-TGA ATC GCT CAC AGT TAT T-3' (SEQ. ID. NO.:69; LPL HindIII:2251U19);
 5'-ATC GCT CAC AGT TAT TCA G-3' (SEQ. ID. NO.:70; LPL HindIII:2254U19);

5'-TCG CTC ACA GTT ATT CAG T-3' (SEQ. ID. NO.:71; LPL HindIII:2255U19);
 5'-CGC TCA CAG TTA TTC AGT G-3' (SEQ. ID. NO.:72; LPL HindIII:2256U19);
 5'-AAT CCC AGC ACA TTT AGT AT-3' (SEQ. ID. NO.:73; LPL HindIII:2507L20);
 5'-ACT ATA GTT TGC AAA ATC CC-3' (SEQ. ID. NO.:74; LPL HindIII 2521L20);
 5'-TGA GAG CTG GGA TTA GAA-3' (SEQ. ID. NO.:75; LPL HindIII:2273U18);
 5'-GAG AGC TGG GAT TAG AAG T-3' (SEQ. ID. NO.:76; LPL HindIII:2274U19);
 5'-AGA GCT GGG ATT AGA AGT C-3' (SEQ. ID. NO.:77; LPL HindIII:2275U19);
 5'-AAT CCC AGC ACA TTT AGT AT-3' (SEQ. ID. NO.:78; LPL HindIII:2507L20); and
 5'-CCC ACC CAT GTG TAC CCA TA-3' (SEQ. ID. NO.:79; LPL HindIII:2446U20).

10 Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS: 1-32 or 35-79 can also be used to amplify nucleic acids comprising the normal locus of the *HindIII* recognition site in *LPL* intron 8. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:1-32 and 35-79 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:1-32 and 35-79 and additional nucleotides adjacent to the position(s) based upon the Nickerson reference sequence.

15 Other primer sequences are useful for amplifying nucleic acid sequences including the (TTTA)_n tetranucleotide repeat region in intron 6. These include SEQ. ID. NOS.:33 and 34, described above and the following primer sequences (designation includes the nucleotide position within the Nickerson reference sequence in Genbank accession AF050163, e.g., 4644 or 4934, at which the 5'-terminus of the primer sequence begins if it is an upper [“U”, i.e., forward] primer, or the position complementary to a position 20 in AF050163 at which its 3'-terminus ends if it is a lower [“L”, i.e., reverse] primer, and primer length, e.g., 24 bases):

5'-CTG GAC AAG AGT CTA AAG CAG CAT-3' (SEQ. ID. NO.:82; LPL:4644U24);

5'-GAA TCG CTT GAA CCG GAA AG-3' (SEQ. ID. NO.:83; LPL:4934L20);

5'-ACC ATC AGT CTT AAG AGA TCT GTG-3' (SEQ. ID. NO.:84; LPL:4934L24);

25 5'-CAC AGA TCT CTT AAG ACT GAT GGT-3' (SEQ. ID. NO.:85; LPL:4693L24);
 5'-TTT TTC ACC TGG ACA AGA GT-3' (SEQ. ID. NO.:86; LPL:4636U20);

5'-GGG TAA CTG AGC GAG ACC GT-3' (SEQ. ID. NO.:87; LPL:4870L20);

5'-TTC ACC TGG ACA AGA GTC TA-3' (SEQ. ID. NO.:88; LPL:4639U20);

5'-GCT TGA ACC GGA AAC-3' (SEQ. ID. NO.:89; LPL:4934L15);

30 5'-TCA CCT GGA CAA GAG TCT AA-3' (SEQ. ID. NO.:90; LPL:4640U20);
 5'-CTC CAG CCT GGG TAA CT-3' (SEQ. ID. NO.:91; LPL:4882L17); and
 5'-ACA AGA GTC TAA AGC AGC AT-3' (SEQ. ID. NO.:92; LPL:4648U20).

Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS:33 and 34 or 82-92 can also be used to amplify nucleic acids comprising the (TTTA)_n tetranucleotide repeat region in *LPL* intron 35 6. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:33-34 and 82-92 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:33-34 and 82-92 and additional nucleotides adjacent to the position(s) based upon the Nickerson reference sequence.

Other primer sequences are useful for amplifying nucleic acid sequences in exon 10 and the 3'-UTR. These include the following primer sequences (SEQ. ID. NOS.:95-106) (designation includes the

nucleotide position within the Oka reference sequence in GenBank accession X52978 X53518, e.g., 2564, at which the 5'-terminus of the primer sequence begins if it is an upper [“U”; i.e., forward] primer; or the position complementary to a position in X52978 X53518 at which its 3'-terminus ends if it is a lower [“L”; i.e., reverse] primer; and primer length, e.g., 22 bases):

- 5 5'-ATG AAA AGA GCA TAT GGT GGT T-3' (SEQ. ID. NO.:95; LPL 3' end Oka 2564U22);
- 5'-TGG CCC AGG TAT ACA TAT GTA ACT A-3' (SEQ. ID. NO.:96; LPL 3' end Oka 2845L25);
- 5'-GGC CCA GGT ATA CAT ATG TAA CTA A 3' (SEQ. ID. NO.:97; LPL-3' end Oka 2844L25);
- 5'-TGA AAA GAG CAT ATG GTG GTT C 3' (SEQ. ID. NO.:98; LPL-3' end Oka 2565U22);
- 5'-GAA AAG AGC ATA TGG TGG TTC-3' (SEQ. ID. NO.:99; LPL 3' end Oka 2566U21);
- 10 5'-GCC CAG GTA TAC ATA TGT AAC TAA C-3' (SEQ. ID. NO.:100; LPL 3' end Oka 2843L25);
- 5'-AAA AGA GCA TAT GGT GGT TC-3' (SEQ. ID. NO.:101; LPL 3' end Oka 2567U20);
- 5'-GGT TCT CTC AGC TCC CAG CCA ACA A-3' (SEQ. ID. NO.:102; LPL 3' end Oka 2582U25);
- 5'-AGC ACA CCA ACA TGG CCC AGG TA-3' (SEQ. ID. NO.:103; LPL 3' end Oka 2869L23);
- 5'-CTC AGC TCC CAG CCA ACA ACC AGT C-3' (SEQ. ID. NO.:104; LPL 3' end Oka 2588U25);
- 15 5'-CAG CAC ACC AAC ATG GCC CAG GTA-3' (SEQ. ID. NO.:105; LPL 3' end Oka 2859L24); and
- 5'-AGC TCC CAG CCA ACA ACC AGT CTC G-3' (SEQ. ID. NO.:106; LPL 3' end Oka 2591U25).

Any 15- to 28-mer primer sequence overlapping any of SEQ. ID. NOS: 95-106 with respect to its position on the Oka reference sequence can also be used to amplify nucleic acids comprising *LPL* exon 10 and the 3'UTR. The primer sequence can overlap the entire sequence of any of SEQ. ID. NOS.:95-106 or can overlap at one or more contiguous nucleotide positions of any of SEQ. ID. NOS.:95-106 and additional nucleotides adjacent to the position(s) based upon the Oka reference sequence.

The present invention also relates to a primer set for detecting a genetic predisposition for non-responsiveness to statin drug treatment in a human. The primer set functions to initiate nucleic acid synthesis in PCR from both the 5' and 3' ends of a nucleic acid template comprising a non-coding or untranslated region of the 3' end of the human *LPL* gene. The primer set comprises any two suitable oligonucleotide primers of the present invention, as described herein, as long as the primer set includes both a forward (upper or “U”) and a reverse (lower or “L”) primer.

For example, a preferred primer set for amplifying nucleic acids comprising the normal locus of the *Hind*III recognition site in intron 8 of *LPL* includes any pair lower and upper primers from among SEQ. ID. NOS.:1-32 or 35-79 (described above), or primer sequences overlapping any of them with respect to the Nickerson reference sequence. A most preferred set of primers is reverse (lower) primer SEQ. ID. NO.:1 and forward (upper) primer SEQ. ID. NO.:2.

Additional primer sets that are useful for amplifying the region of the (TTTA)_n tetranucleotide repeat include any pair of lower and upper primers from among SEQ. ID. NOS.:33-34 and 82-92 (described above), or primer sequences overlapping any of them with respect to the Nickerson reference sequence. A most preferred embodiment of a primer set for detecting the presence of the (TTTA)_n 4 allele includes primers comprising SEQ. ID. NOS.:33 and 34.

Additional primer sets that are useful for amplifying exon 10 and the 3'UTR include any pair of

lower and upper primers from among SEQ. ID. NOS.:95-106 (described above), or primer sequences overlapping any of them with respect to the Oka reference sequence.

The present invention also relates to a genetic testing kit for detecting in a human subject a genetic predisposition for non-responsiveness to statin drug treatment. The genetic testing kit is a ready assemblage of materials for facilitating the amplifying of nucleic acids from a human subject comprising a nucleotide sequence from a non-coding or untranslated region of the 3' end of the human *LPL* gene and/or analyzing amplification products thereof. A genetic testing kit of the present invention contains at least one oligonucleotide primer of the present invention and preferably comprises a primer set of the present invention, as described above, together with instructions for the practice of the present method. The materials or components assembled in the genetic testing kit are provided to the practitioner stored in any convenient and suitable way that preserves their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures.

Another preferred embodiment of the genetic testing kit incorporates an array of oligonucleotide primers specific for single nucleotide polymorphisms in the human nucleotide sequence of the 3' end of *LPL*, particularly of non-coding or untranslated regions, preassembled in a "DNA chip" (or "gene chip") configuration for facilitating the amplifying of nucleic acids and the analyzing of amplification products. (E.g., J.G. Hacia *et al.*, *Enhanced high density oligonucleotide array-based sequence analysis using modified nucleoside triphosphates*, Nucleic Acids Res. 26(2):4975-82 [1998]; R.W. Wallace, *DNA on a chip: serving up the genome for diagnostics and research*, Mol. Med. Today 3(9):384-89 [1997]; T. Pastinen *et al.*, *Minisequencing: a specific tool for DNA analysis and diagnostics on oligonucleotide arrays*, Genome Res. 7(6):606-14 [1997]; M.T. Cronin *et al.*, *Cystic fibrosis mutation detection by hybridization to light-generated DNA probe arrays*, Hum. Mutat. 7(3):244-55 [1996]; A.C. Pease *et al.*, *Light-generated oligonucleotide arrays for rapid DNA sequence analysis*, Proc. Natl. Acad. Sci. USA 91(11):5022-26 [1994]; E.M. Southern *et al.*, *Arrays of complementary oligonucleotides for analyzing the hybridisation behaviour of nucleic acids*, Nucleic Acids Res. 22(8):1368-73 [1994]).

The skilled practitioner will appreciate that homozygosity for a mutation in a non-coding or untranslated region of the 3' end of the human *LPL* gene, such as the *Hind*III 2/2 or (TTTA)_n 4/4 genotypes, is a risk factor for atherosclerotic stenosis in coronary artery disease independent and additive to the use of statin drugs to reduce LDL. For example, the effect of the *LPL* *Hind*III 2/2 genotype on atherosclerotic graft worsening is of the same magnitude as the use of moderate rather than aggressive drug therapy to lower LDL. Such a genotype apparently does not act via an effect on lipid levels, nor the amount of drug needed to achieve lower levels. However, it is associated with a modest effect on blood pressure.

Using the methods, primers, primer sets, and genetic testing kits of the present invention for detecting a genetic predisposition in a human for non-responsiveness to statin drug treatment for coronary artery disease, the practitioner can identify patients homozygous for a variant allele in a non-coding or

untranslated region of the 3' end of LPL, for example those with the *HindIII* 2/2 or (TTTA)_n 4/4 genotype. These patients are predisposed to develop atherosclerotic progression despite their compliance with aggressive lipid lowering therapy with lovastatin or other statin class drugs.

A high level of LDL-C is an important risk factor of heart disease and atherosclerosis, but it is not the sole risk factor. The present invention provides the practitioner a valuable tool for better characterizing individual patients and identifying those patients likely to need individualized alternative interventions other than LDL-C lowering therapy with statin class drugs. For example, direct blood pressure lowering therapy may be indicated for patients identified as homozygous for a variant genotype in accordance with the present invention, because they tend to have blood pressures at the high end of the normal range. Such treatment can include, for example, angiotensinogen converting enzyme (ACE) inhibitors or Ca²⁺ channel blockers. Alternatively, beta blockers, diuretics, or a combination of modalities can be a more appropriate blood pressure lowering therapy for a given patient. Blood pressure lowering in conjunction with aspirin treatment can prevent heart disease in some patients. (See L. Hansson *et al.*, *Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principle results of the hypertension Optimal Treatment (HOT) randomised trial*, *Lancet* 351(9118):1755-62 [1998]; *Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischemic heart disease in men at increased risk*, *Lancet* 351(9098):233-41 [1998]).

For patients identified as homozygous for a variant allele in accordance with the present invention, the practitioner can look at a variety of other known or suspected atherogenic risk factors, beyond LDL-C levels, that may be amenable to treatment in an individual patient. For example, small LDL particle sizes may be amenable to treatment with fibric acid-derivative drugs, e.g., lopid, or high dose niacin. (See J.R. Guyton *et al.*, *Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia*, *Am. J. Cardiol.* 82(6):737-43 [1998]). High Lp(a) levels may be treatable with niacin, or estrogen replacement therapy in women or testosterone replacement in men.

For some patients identified as homozygous for a variant allele in accordance with the present invention, such as the *HindIII* 2/2 or (TTTA)_n 4/4 genotype, the practitioner can appropriately focus on altering atherogenic life style factors such as diet, smoking, and exercise. (E.g., see J.C. LaRosa, *The role of diet and exercise in the statin era*, *Prog. Cardiovasc. Dis.* 41(2):137-50 [1998]).

In view of the substantial cost of statin drugs, a secondary benefit to be derived from identifying patients having a genetic predisposition to non-responsiveness to statin drug treatment, for coronary artery disease or high blood pressure, is the cost savings to patients and health care systems that can be gained by relying on more individually suited alternative treatments instead of statin treatment regimens, for those individuals for whom statins are likely to be ineffective. (See D.M. Huse *et al.*, *Cost-effectiveness of statins*, *Am. J. Cardiol.* 82(11):1357-63 [1998]; P.N. Durrington, *Can we afford to treat hyperlipidaemia as we should? Strategies for rational treatment*, *Atherosclerosis* 139(Suppl. 1):S1-5 [1998]; J.A. Farmer, *Economic implications of lipid-lowering trials: current considerations in selecting a statin*, *Am. J.*

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Cardiol. 82(6A):26M-31M [1998]).

By using the methods, primers, primer sets, and genetic testing kits of the present invention, the practitioner can better individualize the treatment and improve the care of patients with coronary artery disease.

5 The detailed examples which follow describe the genetic association between variant alleles in non-coding or untranslated regions of the 3' end of the human *LPL* gene and atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment. These examples are intended merely to illustrate and in no way limit the present invention.

EXAMPLES

10 Genetic link between mutant *LPL* genotypes and phenotypic atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment.

The following examples describe further data and analyses that support a genetic association between the *LPL* HindIII 2/2 or (TTTA), 4/4 genotypes and a phenotype of atherosclerotic stenosis in coronary artery disease that is non-responsive to statin drug treatment.

15 Example 1. Study Design

A genetic association study was conducted by a within-case comparison ancillary to the Post Coronary Artery Bypass Graft Trial. (The Post Coronary Artery Bypass Graft Trial Investigators. *The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in saphenous-vein coronary-artery bypass grafts*; N. Engl. J. Med. 336:153-62

20 [1997]). A two stage design was followed. First, EBV-transformed lymphoblastoid cell lines were established for subjects from the Los Angeles (L.A.) cohort providing a permanent source of DNA for testing hypotheses related to atherosclerosis-related candidate genes. Then, significant results were tested in a second stage by genotyping all available subjects in the post-CABG trial using DNA isolated from whole blood shipped to Cedars-Sinai Medical Center from the other participating centers.

25 Participants were randomly assigned, following a two by two design, to receive 1) lovastatin therapy to lower the LDL-cholesterol level to within the range of 93-97 mg/dl (aggressive treatment group) or 132-136 mg/dl (moderate treatment group), and 2) placebo or low-dose warfarin (Post-CABG, 1997). Coronary angiograms of 1351 subjects at enrollment and an average of 4.3 years later were compared using a quantitative assessment of the severity of graft stenosis. Graft worsening was defined as a decrease in 30 lumen diameter of 0.6mm or more. The percentage of subjects with worsening of one or more grafts was 39% in the aggressive treatment group compared to 51% in the moderate treatment group ($p<0.001$) and the mean percentage of grafts per patient showing worsening was 27% in the aggressive treatment group compared to 39% in the moderate treatment group ($p<0.001$).

35 These results demonstrated the efficacy of lowering LDL-cholesterol levels with statin drug treatment in reducing the risk of graft worsening in most CABG patients. No effect of the warfarin

treatment on graft worsening was observed.

Example 2. Subjects

A total of 1351 subjects from seven clinical centers throughout North America were included in the clinical trial and all were eligible as participants. Genetic material was received from 891 subjects who

5 were included in this ancillary study. Inclusion criteria for the clinical trial were: bypass surgery 1-11 years prior to the study; an LDL-cholesterol level of 130-175 mg/dl; and at least one patent vein graft as determined by angiography. Subjects were excluded if there was: (a) the likelihood of revascularization or death within the study period of 5 years; (b) unstable angina or myocardial infarction within six months before the start of the trial; (c) severe angina; (d) heart failure; or (e) contraindications to the study
 10 medications. *Id.* Subjects were randomly assigned in a two by two factorial design for treatment to lower LDL-cholesterol levels aggressively (target LDL 93-97 mg/dl) or moderately (target LDL 132-136 mg/dl) with lovastatin and cholestyramine if needed, and for treatment with either placebo or warfarin sufficient to maintain an international normalized ratio of less than 2. *Id.* Graft worsening was determined by comparing the initial angiogram at enrollment with a follow-up angiogram repeated an average of 4.3 years
 15 later. "Worsening" was defined as a reduction in diameter ≥ 0.6 mm in diameter. "Subjects with worsening" were defined as those subjects with one or more grafts showing worsening.

Example 3. Data Collection

Questionnaire data regarding demographics, family and medical history, and angiographic and clinical data were collected as part of the post-CABG trial. Additional family history data were collected
 20 from 891 subjects in the genetic ancillary study.

Example 4. DNA

During years 2-3 of the clinical trial, cell lines from 224 subjects in the L.A. cohort were established by transformation of peripheral blood lymphocytes with Epstein-Barr virus (EBV). (M. A. Anderson and J.F. Gusella, *Use of cyclosporin-A in establishing Epstein-Barr virus transformed human*

25 *lymphoblastoid cell lines.* *In Vitro* 21:856-58 [1984]; S. Pressman and J. I. Rotter, *Epstein-Barr virus transformation of cryopreserved lymphocytes, prolonged experience with technique, letter to the editor*, *Am. J. Hum. Genet.* 49:467 [1991]). During years 4-5, whole blood was collected from an additional 667 subjects from the other centers. Thus, DNA was available from a total of 891 subjects. DNA was isolated following standard protocols. (B.G. Herrman and A. Frischauf, *Isolation of Genomic DNA, Methods in*
 30 *Enzymology* 152:180-83 [1987]).

Example 5. Genotyping

Conventional agarose gel techniques were used to genotype the LA cohort for the biallelic *LPL Hind*III polymorphism following Heizmann *et al.* (C. Heizmann *et al.*, *RFLP for the human lipoprotein*

lipase (LPL) gene: HindIII, Nucleic Acids Res. 15:6763 [1987]). DNA samples from the remaining subjects were genotyped for this polymorphism as well as four additional LPL polymorphisms using fluorescent semi-automated technology. In Figure 1(a), the location of polymorphisms in the LPL gene was assembled from information in GenBank, accession numbers G187209, G34390, M76722, and

5 M76723, and other published sources. (F. Mailly *et al.*, *A common variant in the gene for lipoprotein lipase (asp9-asn): functional implications and prevalence in normal and hyperlipidemic subjects*, Arterioscler. Thromb. Vasc. Biol. 15:468-78 [1995]; P.W.A. Reymer *et al.*, *A lipoprotein lipase mutation (asn291ser) is associated with reduced HDL cholesterol levels in premature atherosclerosis*, Nat Genet 1995;10:28-34 [1995]; C. Heizmann *et al.*, *DNA polymorphism haplotypes of the human lipoprotein 10 lipase gene: possible association with high density lipoprotein levels*, Hum. Genet. 86:578-84 [1991]; G. Zuliani and H.H. Hobbs, *Tetranucleotide repeat polymorphism in the LPL gene*, Nucleic Acids Res. 18:4958 [1990]).

Marker genotypes were determined using a PCR with primers listed below as recommended by the manufacturer of Ampli-Taq Gold (Perkin Elmer, Foster City, CA) in a Perkin Elmer 9600 15 thermocycler. (All PCR runs began with 95° for 10 min. to activate the polymerase). After digestion with the appropriate restriction enzyme, PCR products for each subject were pooled from all five genotyping reactions and run together on 6% Long Ranger gels in a semi-automated DNA sequencer (ABI 373 DNA sequencer, Applied Biosystems, Foster City, CA) with gel processing using Genescan and Genotyper software.

20 **D9N (exon 2).** The assay of Mailley *et al.* (1995) was redesigned using the sequence in GenBank accession G187209 so that the forward primer (5'-Hex-*ACT CCG GGA ATG AGG T*; SEQ. ID. NO.:107) carried the detection dye and the reverse primer (CCA GAA AGA AGA GAT TTT GTC; SEQ. ID. NO.:108) introduced a *SalI* restriction site if the PCR fragment carried the mutated D9N allele, and resulted in a 98 bp fragment for the D allele (1 allele) and a 77 bp fragment for the N allele (2 allele) after 25 *SalI* digestion. PCR conditions were 35 cycles of 94°C 30 sec, 46°C 30 sec, 72°C 30 sec.

N291S (exon 6). The procedure of Reymer *et al.* (1995) was followed with Hex added to the forward primer. PCR conditions were 35 cycles of 94°C 30 sec, 60°C 30 sec, 72°C 30 sec. The reverse primer introduces a partial *RsaI* site so that the N allele gave a 242 bp fragment (1 allele) and the S allele gave a 218 bp fragment (2 allele) after the *RsaI* digestion.

30 **PvuII (intron 6).** The assay of Li *et al.* (S. Li *et al.*, *PvuII RFLP at the human lipoprotein [LPL] gene locus*, Nucleic Acids Res. 16:2358 [1988]) was redesigned using the sequence in GenBank accession number g34390 so that the resulting fragments would run less than 350 bp in size on the ABI 373. The forward primer was 5'-Tet-CTG CTT TAG ACT CTT GTC CAG GTG (SEQ. ID. NO.:109) and the reverse primer was 5'-GGG TTC AAG GCT CTG TCA GTG TCC (SEQ. ID. NO.:110). PCR conditions

were 35 cycles of 94°C 30 sec, 55°C 30 sec, 72°C 30 sec. A 155 bp fragment was detected if the *Pvu*II site was present (1 allele) and a 282 bp fragment was detected if the *Pvu*II site was absent (2 allele).

(TTTA)_n (intron 6). The procedure of Zuliani and Hobbs (1990) was followed using FAM-labeled GZ-15 primer (5'-CCT GGG TAA CTG AGC GAG ACT GTG TC-3'; SEQ. ID. NO.:33) and GZ-14 primer (5'-

5 ATC TGA CCA AGG ATA GTG GGA TAT A-3'; SEQ. ID. NO.:34). PCR conditions were 35 cycles of 94°C 30 sec, 68°C 3 min. Allele 1 ran at a size of 119 bp, 2 at 123 bp, 3 at 127 bp, 3 at 127 bp, 4 at 131 bp, and 5 at 135 bp.

*Hind*III (intron 8). The assay of Heinzmann *et al.* (1987) was used for stage 1 and then was redesigned for stage 2 using the sequence in GenBank accession numbers M76722 and M76723. Reverse primer was

10 5'-Fam-GCA TCT GCC TTC AGC TAG ACA TTG (SEQ. ID. NO.:1) and forward primer was 5'-TCT TCC AGA AGG GTG AGA TTC CAA (SEQ. ID. No.:2). PCR conditions were the same as described above for *Pvu*II. Using this primer set of SEQ. ID. NOS.:1 and 2, a 228 bp fragment was detected if the *Hind*III restriction site was present (1 allele) and a 330 bp fragment if absent (2 allele).

Example 6. Statistical Methods

15 Differences in baseline characteristics between treatment groups and between genotype groups were tested by one-way analysis of variance or Chi-square tests. Log-transformed HDL and TG values were used to perform all statistical analyses in order to adjust for their skewed distributions, but are presented in the tables as untransformed means \pm SE. The association between graft worsening and LPL genotype was tested by Chi-square test. Mantel-Haenszel statistics were used for testing the interactions between genotypes and treatment groups. The proportion of grafts showing worsening per subject was used as the quantitative measurement of graft worsening, and multiple regression was performed to for this proportion as a function of genotype and treatment group to identify predictors. Adjusted variables for this trait included age, gender, body mass index, smoking status, number of years since CABG, systolic and diastolic blood pressure, current medicine use, and family history as listed in Table 1. All statistical analyses were carried out with SAS software (version 6.12, SAS Institute, Cary, NC).

Example 7. Baseline Characteristics

Table 1(a) compares the baseline characteristics of the subjects in the aggressive and moderate drug treatment groups. Minor differences were observed in the percent of subjects with a history of stroke, percent using diabetic therapies, systolic and diastolic blood pressures, and baseline LDL levels. The highly significant difference in the steady state levels of total cholesterol and LDL-cholesterol between these two groups reflects the effect of the drug treatment. As shown in Table 1(b), significant differences were observed between the 891 subjects in the genetic study and the 460 subjects who were not included: frequency of prior myocardial infarction, 46% vs. 55%, $p=0.001$; smoking 9% vs. 14%, $p=0.005$; mean years from CABG to enrollment, 4.7 vs. 5.2 years, $p<0.001$; and aspirin use, 79% vs. 69%, $p=0.001$.

Table 1. Characteristics of Subjects by Treatment Group (1a) or by Inclusion or Exclusion in Genetic Study (1b).

Characteristic

1a: By Treatment Group

1b: By Inclusion or Exclusion
in Genetic Study

5

	Aggressive (N=430)	Moderate (N=406)	p	Included (N=891)	Excluded (N=460)	p
--	-----------------------	---------------------	---	---------------------	---------------------	---

10 Age (yr, mean(SE)

62.2 \pm 0.4

62

61

Caucasian (%)

94

94

96

Male (%)

91

92

93

15 Body mass index (kg/m², mean(SE))27.2 \pm 0.227.3 \pm 0.2

Current smoking (%)

15

9

9

0.005

History of myocardial infarction (%)

45

48

46

0.001

15 Time between CABG and enrollment

(yr, mean(SE))

4.7 \pm 0.1

4.7

5.2

<0.001

Ejection fraction (%):

56

57

57

Family history of (%):

20 Coronary artery disease

76

70

70

Diabetes

36

33

*

Hypertension

58

56

Peripheral vascular disease

19

21

Stroke

41

48

0.05

25 Current medications (%):

Aspirin

82

77

79

0.001

Beta-blocker

25

24

24

Calcium-channel blocker

27

21

23

Insulin or oral antidiabetic agent

10

6

8

30 Thiazide diuretic

12

9

11

Systolic blood pressure

(mmHg, mean(SE))

134.4 \pm 0.8133.0 \pm 0.9

0.03

Diastolic blood pressure

(mmHg, mean(SE))

79.3 \pm 0.479.7 \pm 0.4

0.02

35 Baseline lipid levels (mg/dl):†

Total cholesterol

227.3 \pm 1.2227.4 \pm 1.3

226.9

226.4

LDL cholesterol

156.1 \pm 1.0155.3 \pm 1.0

0.04

155.8

HDL cholesterol

39.3 \pm 0.539.5 \pm 0.5

39.4

39.0

Triglycerides

160.3 \pm 73.2162.2 \pm 3.7

161.1

157.6

40 Steady state lipid levels (mg/dl):

Total cholesterol

172.6 \pm 1.6209.5 \pm 1.6

0.001

LDL cholesterol

97.1 \pm 1.3133.0 \pm 1.3

0.001

HDL cholesterol

44.9 \pm 0.643.7 \pm 0.6

44.3

42.0

Triglycerides

157.4 \pm 4.5164.5 \pm 4.4

163.1

160.6

1000/0/6/2358660

Drug treatment groups and included/excluded in genetic study groups were compared by the analysis of variance
 Blank p values were nonsignificant.

For the *LPL HindIII* genotype, "1" indicates the presence, "2" the absence, of the restriction site in intron 8.
 Complete data for every category in this table was available for 836 of the 891 subjects in this study.

5 *Comparable family history data is unavailable on subjects that were not included in the genetic study
 and so these groups cannot be compared for these characteristics

† Values listed are those measured most recently before enrollment.

To convert cholesterol values to mmol/l, multiply by 0.02586;
 to convert triglyceride values to mmol/l, multiply by 0.01129.

10 **Example 8. *LPL HindIII* and Graft Worsening in L.A. Cohort**

Genotyping of the L.A. cohort for the *LPL HindIII* polymorphism demonstrated that the proportion of subjects with graft worsening increased with the number of *HindIII* 2 alleles: 42% in those with no *HindIII* 2 allele, 54% in those with one, and 72% in those with two (χ^2 2x3 test of association, $p=0.05$). Further, the percent of grafts showing worsening was calculated per subject and 15 the mean of this percentage also increased with the number of *LPL HindIII* 2 alleles, with 22% in the subjects with *HindIII* 1/1, 31% in subjects with 1/2, and 53% in subjects with 2/2 (analysis of variance, $p=0.001$).

Example 9. *LPL HindIII* and Graft Worsening in All Subjects

With this result, the remaining 667 subjects were genotyped. A comparison of the percent of 20 subjects with graft worsening for the two *LPL HindIII* genotypes for all 891 subjects is shown in Table 2. A significant difference in the percent of subjects showing graft worsening was observed between those with the *LPL HindIII* 2/2 genotype compared to those with the *LPL HindIII* 1/1 and 1/2 genotypes combined; 58% of those with the *LPL HindIII* 2/2 genotype exhibited worsening compared with 42% of those with either 1/1 or 1/2 (odds ratio=1.9, 95% confidence interval 1.2-3.2, $p=0.011$).
 25 The mean proportion of grafts showing worsening per subject was also significantly increased for those with the *LPL HindIII* 2/2 genotype (40% for *HindIII* 2/2 compared with 27% for *LPL HindIII* 1/1 and 1/2; $p=0.0066$). There were no significant differences in graft worsening between subjects with the *LPL HindIII* 1/1 and 1/2 genotypes.

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Table 2. Graft Worsening and *LPL HindIII* Genotype.

Phenotype	<i>LPL HindIII</i> Genotype		p value
	2/2 (N=65)	1/1 & 1/2 (N=723)	
Subjects with Worsening (%)	58	41	0.011
Mean Grafts with Worsening/Subject (%)	40	27	0.0066

“Subjects with worsening” defined as subjects with one or more grafts showing worsening, defined as a decrease (0.6 mm in vessel diameter;

“mean grafts with worsening/subject” defined as the mean number of grafts showing

10 “Subjects with worsening” were compared using the Chi square test of association.

“mean grafts with worsening/subject” were compared using analysis of variance.

For the *LPL HindIII* genotype, “1” indicates the presence, “2” the absence, of the restriction site in intron 8.

Example 10. *LPL* Genotypes and Graft Worsening

15 Four additional *LPL* polymorphisms were tested for association with graft worsening in the entire genetic study cohort (Fig. 1). Complete worsening data were available for 792 subjects; complete genotyping data for each marker represented in Figure 1: D9N (exon 2; Mailley *et al.* [1995], N291S (exon 6; Reymer *et al.* [1995]), *PvuII* (intron 6; “1”=site is present, “2”=site is absent; S. Li *et al.* [1988]), (TTTA)_n (intron 6; allele 1 is 119 bp, 2 is 123 bp, 3 is 127 bp, 4 is 131 bp, 5 is 135 bp; D.-

20 A. Wu *et al.*, Quantitative trait locus mapping of human blood pressure to a genetic region at or near the lipoprotein gene locus on chromosome 8p22, *J. Clin. Invest.* 97:2111-18 [1996]), *HindIII* (intron 8; “1”= site is present, “2”=site is absent; C. Heizmann *et al.* [1987]). A designation of “X” is an abbreviation for “other” genotypes. The percent of subjects with graft worsening is the percent of subjects with one or more grafts showing a reduction in diameter ≥ 0.6 mm.

25 Only the (TTTA)_n and *HindIII* polymorphisms were significantly associated with graft worsening by the Chi square test of association. There was no association between graft worsening and the functional D9N and N291S polymorphisms and also no association with the *PvuII* polymorphism. In contrast, the 4/4 genotype of the (TTTA)_n polymorphism was associated with graft worsening: 63% of (TTTA)_n 4/4 subjects had worsening of one or more grafts compared to 43% of subjects with other (TTTA)_n genotypes (OR=2.2, 95%CI 1.1-4.6; p=0.027). The (TTTA)_n 4 allele was found to be in strong linkage disequilibrium with the *HindIII* 2 allele (p<0.001, data not shown). Consequently, the combined genotype of (TTTA)_n 4/4 and *HindIII* 2/2 was also associated with graft worsening at a significance level similar to the (TTTA)_n 4/4 or *HindIII* 2/2 genotypes alone.

30 Graft worsening was significantly associated with the *LPL HindIII* 2/2 genotype and tetranucleotide (TTTA)_n 4/4 polymorphisms, both individually and together. The *LPL HindIII* 2/2

polymorphism did not appear to be acting through any lipid variables, but was associated with significant differences in systolic and diastolic blood pressure.

In contrast, no associations between clinical endpoints and the *LPL* D9N, N291S, or *Pvu*II polymorphisms were observed, indicating that the as yet unknown functional mutation associated with 5 graft worsening is in linkage disequilibrium with the (TTTA)_n and *Hind*III polymorphisms, and thus resides in the 3'-end of the *LPL* gene.

Multiple regression analysis demonstrated that there were no differences in the baseline or steady-state serum lipid values, or the response to lipid-lowering therapy between those subjects with the *LPL Hind*III 2/2 genotype and those with the other *Hind*III genotypes (i.e., 1/1 or 1/2). While the 10 present invention is not committed to any particular mechanism, this observation indicates that the *LPL* polymorphism does not act through an effect on LDL-cholesterol. This result is congruent with that of Peacock *et al.* (1992) who observed an association between the *LPL Hind*III 2 allele and the angiographic severity of atherosclerosis without observing concomitant differences in the mean fasting serum lipid levels in a comparison of young myocardial infarction survivors and age-matched controls.

15 Some significant differences in important risk factors for atherosclerosis were observed among the group of subjects in the genetic study described herein, including: the frequency of prior myocardial infarction, smoking, aspirin use, and mean years from CABG to enrollment. But if a survival bias occurred, it would lead to an underestimate of the effect of the *LPL Hind*III 2/2 genotype on the risk of graft worsening. Further, in the 891 subjects for which DNA was available, there were no important 20 differences between the aggressive and moderate treatment groups as to the effect of *Hind*III 2/2 upon responsiveness to statin treatment, as described below.

Example 11. Characteristics of the *Hind*III 2/2 Genotype Group

To investigate potential mechanisms for the association between the *LPL Hind*III 2/2 genotype and graft worsening, baseline characteristics and response of the subjects to the lipid-lowering action of 25 lovastatin were compared between subjects (Table 3). There were no differences observed between the baseline values for total cholesterol, HDL-cholesterol, and triglycerides. However, a small difference in LDL-cholesterol was observed, 159.6 ± 2.1 mg/dl for subjects with *Hind*III 2/2 compared with 155.0 ± 0.7 for 1/1 and 1/2, $p=0.04$. There were no differences in any of the lipid values attained as a result of drug treatment during the trial, nor was the amount of drug necessary to achieve target lipid 30 values significantly different between the two genotype groups. In contrast to the essentially similar lipid profile of the *LPL Hind*III genotype groups, the *Hind*III 2/2 subjects did vary consistently on one set of physiologic parameters. They had a higher average blood pressure, systolic pressure 138.6 ± 2.1 mmHg vs. 133.7 ± 0.6 for subjects with other genotypes, $p=0.03$; and diastolic pressure 82.1 ± 1.0 mmHg vs. 79.4 ± 0.3 for subjects with other genotypes, $p=0.02$.

35 Multiple regression analysis showed that graft worsening or stenosis was associated with an interaction between the *LPL* genotype and blood pressure. The *LPL Hind*III 2/2 effect on blood

pressure observed here probably has little effect in normal subjects. However, in the presence of ongoing vascular pathology or clinical atherosclerosis, a modest change due to a genetic factor might exert a greater effect. For example, while a blood pressure increase within the normal range has little effect in the general population, slight increases in blood pressure are a significant risk factor for nephropathy in type I diabetes, such that blood-pressure lowering intervention is recommended for some normotensive type I diabetic subjects. (J. Barzilay *et al.*, *Predisposition to hypertension: risk factor for nephropathy and hypertension in IDDM*, *Kidney Int.* 42:723-30 [1992]; E.J. Lewis *et al.*, *The effect of angiotensin-converting enzyme inhibition on diabetic nephropathy*, *N. Engl. J. Med.* 329:1456-62 [1993]). Thus, for those patients with an unfavorable *LPL* genotype (e.g., *HindIII* 2/2) other therapies may be indicated in addition to or instead of lipid-lowering statin treatment for prevention of atherosclerotic stenosis.

Table 3. Characteristics of Subjects with *HindIII* 2/2 Genotype

Characteristic	<i>LPL HindIII Genotype</i>	
	2/2 (N=74)	1/1 & 1/2 (N=817)
	62.2±0.8	61.7±0.3
Age (yr, mean(SE))	62.2±0.8	61.7±0.3
Caucasian (%)	97	94
Male (%)	92	92
Current smoking (%)	15	9
Body mass index (kg/m ² ,mean(SE))	26.7±0.4	27.3±0.2
History of myocardial infarction (%)	47	46
Time between CABG and enrollment (yr, mean(SE))	5.0±0.3	4.7±0.1
Ejection fraction (%)	57	57
Family history of (%):		
Coronary heart disease	76	70
Diabetes	36	33
Hypertension	58	56
Peripheral vascular disease	19	21
Stroke	41	45
Current medications (%):		
Aspirin	82	79
Beta-blocker	23	24
Calcium-channel blocker	26	24
Insulin or oral antidiabetic agent	7	8
Thiazide diuretic	7	11
Systolic blood pressure (mmHg, mean(SE)*)	138.6±2.1	133.7±0.6
Diastolic blood pressure (mmHg, mean(SE)*)	82.1±1.0	79.4±0.3
Baseline lipid levels (mg/dl):		
Total cholesterol	230.7±2.7	226.7±0.9
LDL cholesterol*	159.6±2.1	155.0±0.7
HDL cholesterol	41.0±1.1	39.3±0.3
Triglycerides	150.8±7.3	161.7±2.4
Steady state lipid levels (mg/dl): †		
Total cholesterol	191.1±4.8	191.0±1.3
LDL cholesterol	115.1±4.3	114.7±1.1

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	HDL cholesterol	46.7 \pm 1.6	44.1 \pm 0.4
	Triglycerides	151.0 \pm 9.6	163.6 \pm 3.4
	Lipid change (%):		
5	Total cholesterol	16.9 \pm 2.0	15.2 \pm 0.6
	LDL cholesterol	53.5 \pm 6.9	45.1 \pm 1.5
	HDL cholesterol	14.1 \pm 3.1	14.2 \pm 1.0
	Triglycerides	6.0 \pm 5.8	8.2 \pm 2.0
	Mean lovastatin dose required to reach target lipid levels (mg/day):		
10	Aggressive treatment group	37	36
	Moderate treatment group	6.0	6.5

LPL *HindIII* genotype groups were compared by the analysis of variance.

Blank p values were nonsignificant.

For the LPL *HindIII* genotype, "1" indicates the presence, "2" the absence, of the restriction site in intron 8.

15 * p-value less than 0.05.

For the difference between the systolic blood pressures, p=0.03, for the difference between the diastolic blood pressures, p=0.02, for the difference between the LDL cholesterol levels, p=0.04.

† Values listed are those measured most recently before enrollment.

20 To convert cholesterol values to mmol/l, multiply by 0.02586; to convert triglyceride values to mmol/l, multiply by 0.01129.

Example 12. *HindIII* 2/2 Genotype and Statin Drug Treatment

The percent of subjects with graft worsening when stratified by lovastatin treatment group and *LPL HindIII* genotype is shown in Figure 2. Complete *LPL HindIII* genotype data and worsening data were available for 786 subjects.

25 The highest percentage of subjects with worsening were those with the *HindIII* 2/2 genotype assigned to the moderate lipid-lowering treatment group (65%). The lowest percentage of subjects with worsening were those with the *HindIII* 1/1 or 1/2 genotype assigned to the aggressive lipid-lowering treatment group (35%). Within the *LPL HindIII* 1/1 and 1/2 genotype group, the moderate drug treatment group had a significantly higher percent of subjects with graft worsening than the aggressive treatment group, 49% compared with 35%, odds ratio=1.8, 95% confidence interval 1.3 to 2.4, p<0.001. Within the aggressive treatment group, the *LPL HindIII* 2/2 genotype group had a significantly higher percent of subjects with graft worsening, 54% vs. 35%; OR=2.14, 95%CI 1.11-4.11, p=0.023. The effect of genotype on graft worsening, adjusted for treatment, was significant at p=0.006, OR=2.06, 95%CI 1.23-3.43, and the effect of treatment on graft worsening, adjusted for genotype was significant at p=0.001, OR=1.78, 95%CI 1.32-2.4. The combined effect of both the unfavorable *LPL HindIII* genotype with moderate drug treatment yielded an odds ratio of 3.5 for graft worsening, 95%CI 1.4-8.7, p=0.002.

40 Using the proportion of grafts with worsening per subject as the dependent variable, the interactions between factors were tested using multiple regression analysis. After adjustments were made for age, sex, body mass index (BMI), smoking, current medication usage, medical history, and family history, the drug treatment group (p=0.0001) and the interaction between the *LPL HindIII* 2/2 genotype and diastolic blood pressure (p=0.0046) remained significant. No interaction between the

dose of lovastatin required to bring each subject to their target LDL-cholesterol level and the *HindIII* 2/2 genotype was observed.

When subjects were stratified by their *LPL HindIII* genotype and drug treatment group, each factor had a similar effect on graft worsening, with odds ratios of 2.1 and 1.8 respectively. The

5 combined effect of both the unfavorable *LPL HindIII* genotype and moderate lipid-lowering yielded an odds ratio for graft worsening of 3.5 (95%CI 1.4-8.7, p=0.002). This analysis demonstrates that the *LPL HindIII* 2/2 genotype is an independent and additive risk factor for worsening of grafts with an odds ratio of the same magnitude as that for lipid-lowering in the post-CABG trial.

The foregoing examples being illustrative but not an exhaustive description of the

10 embodiments of the present invention, the following claims are presented.

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